The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ US

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference 59138-B-PCT
International application No. PCT/US00/12065	International filing date *(day/month/year)* 3 May 2000	(Earliest) Priority date *(day/month/year)* 3 May 1999

Title of invention
DNA ENCODING SNORF36a AND SNORF36b RECEPTORS

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

SYNAPTIC PHARMACEUTICAL CORPORATION
215 College Road
Paramus, New Jersey 07652
United States of America

Telephone No.: None
Facsimile No.: None
Teleprinter No.: None

State *(i.e. country)* of nationality:	State *(i.e. country)* of residence:
United States of America	United States of America

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BOROWSKY, Beth E.
218 Park Street
Montclair, New Jersey 07042
United States of America

State *(i.e. country)* of nationality:	State *(i.e. country)* of residence:
United States of America	United States of America

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

OGOZALEK, Kristine L.
25 Durand Place
Rochelle Park, New Jersey 07662
United States of America

State *(i.e. country)* of nationality:	State *(i.e. country)* of residence:
United States of America	United States of America

[X] Further applicants are indicated on a continuation sheet.

Sheet No. 2

International application No.
PCT/US00/12065

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet is not to be included in the demand.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

LAKHLANI, Parul P.
304 Spring Valley Road
Paramus, New Jersey 07652
United States of America

State (*i.e. country*) of nationality:	State (*i.e. country*) of residence:
India	United States of America

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

ADHAM, Nika
301 Mastin Place
Ridgewood, New Jersey 07450
United States of America

State (*i.e. country*) of nationality:	State (*i.e. country*) of residence:
United States of America	United States of America

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State (*i.e. country*) of nationality:	State (*i.e. country*) of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State (*i.e. country*) of nationality:	State (*i.e. country*) of residence:

☐ Further applicants are indicated on another continuation sheet.

Sheet No. 3..

International application No.
PCT/US00/12065

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

WHITE, John P.
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, New York 10036
United States of America

Telephone No.: (212) 278-0400

Facsimile No.: (212) 391-0526

Teleprinter No.: None

[ ] Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV STATEMENT CONCERNING AMENDMENTS

The applicant wishes the International Preliminary Examining Authority*

(i) [ ] to start the international preliminary examination on the basis of the international application as originally filed.

(ii) [ ] to take into account the amendments under Article 34 of

[ ] the description (amendments attached).

[ ] the claims (amendments attached).

[ ] the drawings (amendments attached).

(iii) [ ] to take into account any amendments of the claims under Article 19 filed with the International Bureau (a copy is attached).

(iv) [ ] to disregard any amendments of the claims made under Article 19 and to consider them as reversed.

(v) [ ] to postpone the start of the international preliminary examination until the expiration of 20 months from the priority date unless that Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Box No. V ELECTION OF STATES

[X] The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)* except ....

....

....

....

(If the applicant does not wish to elect certain eligible States, the name(s) or country code(s) of those States must be indicated above.)

Sheet No. 4

International application No.
PCT/US00/12065

Box No. VI CHECK LIST

The demand is accompanied by the following documents for the purposes of international preliminary examination:

For International Preliminary Examining Authority use only

			received	not received
1. amendments under Article 34				
description	:	sheets	☐	☐
claims	:	sheets	☐	☐
drawings	:	sheets	☐	☐
2. letter accompanying amendments under Article 34	:	sheets	☐	☐
3. copy of amendments under Article 19	:	sheets	☐	☐
4. copy of statement under Article 19	:	sheets	☐	☐
5. other *(specify)*:	:	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☒ fee calculation sheet
5. ☒ other *(specify)*: Express Mail Certificate of Mailing bearing Express Mail Label #EF299938184US dated 1 December 2000

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

John P. White, Reg. No. 28,678

1 December 2000
Date

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5. below. does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date. the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 12 February 2001 (12.02.01)	
International application No. PCT/US00/12065	**Applicant's or agent's file reference** 59138-B-PCT
International filing date (day/month/year) 03 May 2000 (03.05.00)	**Priority date** (day/month/year) 03 May 1999 (03.05.99)
Applicant BOROWSKY, Beth, E. et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   01 December 2000 (01.12.00)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was
   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No.: (41-22) 740.14.35	Authorized officer S. Mafla Telephone No.: (41-22) 338.83.38

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 59138-B-PCT

Box No. I TITLE OF INVENTION

DNA ENCODING SNORF36a AND SNORF36b RECEPTORS

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

SYNAPTIC PHARMACEUTICAL CORPORATION
215 College Road
Paramus, New Jersey 07652
United States of America

☐ This person is also inventor.

Telephone No. None

Facsimile No. None

Teleprinter No. None

State *(that is, country)* of nationality: United States of America

State *(that is, country)* of residence: United States of America

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

BOROWSKY, Beth E.
218 Park Street
Montclair, New Jersey 07042
United States of America

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: United States of America

State *(that is, country)* of residence: United States of America

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

WHITE, John P.
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, New York 10036
United States of America

Telephone No. (212) 278-0400

Facsimile No. (212) 391-0526

Teleprinter No. None

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. II FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

OGOZALEK, Kristine L.
25 Durand Place
Rochelle Park, New Jersey 07662
United States of America

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: United States of America

State *(that is, country)* of residence: United States of America

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [X] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

LAKHLANI, Parul P.
304 Spring Valley Road
Paramus, New Jersey 07652
United States of America

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: India

State *(that is, country)* of residence: United States of America

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [X] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

ADHAM, Nika
301 Mastin Place
Ridgewood, New Jersey 07450
United States of America

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: United States of America

State *(that is, country)* of residence: United States of America

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [X] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes: at least one must be marked)*:

Regional Patent

- [X] **AP** **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SL** Sierra Leone, **SZ** Swaziland, **TZ** United Republic of Tanzania, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- [X] **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- [X] **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH** and **LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- [X] **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* ....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- [X] **AE** United Arab Emirates
- [X] **AL** Albania
- [X] **AM** Armenia
- [X] **AT** Austria
- [X] **AU** Australia
- [X] **AZ** Azerbaijan
- [X] **BA** Bosnia and Herzegovina
- [X] **BB** Barbados
- [X] **BG** Bulgaria
- [X] **BR** Brazil
- [X] **BY** Belarus
- [X] **CA** Canada
- [X] **CH and LI** Switzerland and Liechtenstein
- [X] **CN** China
- [X] **CR** Costa Rica
- [X] **CU** Cuba
- [X] **CZ** Czech Republic
- [X] **DE** Germany
- [X] **DK** Denmark
- [X] **DM** Dominica
- [X] **EE** Estonia
- [X] **ES** Spain
- [X] **FI** Finland
- [X] **GB** United Kingdom
- [X] **GD** Grenada
- [X] **GE** Georgia
- [X] **GH** Ghana
- [X] **GM** Gambia
- [X] **HR** Croatia
- [X] **HU** Hungary
- [X] **ID** Indonesia
- [X] **IL** Israel
- [X] **IN** India
- [X] **IS** Iceland
- [X] **JP** Japan
- [X] **KE** Kenya
- [X] **KG** Kyrgyzstan
- [X] **KP** Democratic People's Republic of Korea
- [X] **KR** Republic of Korea
- [X] **KZ** Kazakhstan
- [X] **LC** Saint Lucia
- [X] **LK** Sri Lanka
- [X] **LR** Liberia
- [X] **LS** Lesotho
- [X] **LT** Lithuania
- [X] **LU** Luxembourg
- [X] **LV** Latvia
- [X] **MA** Morocco
- [X] **MD** Republic of Moldova
- [X] **MG** Madagascar
- [X] **MK** The former Yugoslav Republic of Macedonia
- [X] **MN** Mongolia
- [X] **MW** Malawi
- [X] **MX** Mexico
- [X] **NO** Norway
- [X] **NZ** New Zealand
- [X] **PL** Poland
- [X] **PT** Portugal
- [X] **RO** Romania
- [X] **RU** Russian Federation
- [X] **SD** Sudan
- [X] **SE** Sweden
- [X] **SG** Singapore
- [X] **SI** Slovenia
- [X] **SK** Slovakia
- [X] **SL** Sierra Leone
- [X] **TJ** Tajikistan
- [X] **TM** Turkmenistan
- [X] **TR** Turkey
- [X] **TT** Trinidad and Tobago
- [X] **TZ** United Republic of Tanzania
- [X] **UA** Ukraine
- [X] **UG** Uganda
- [X] **US** United States of America
  continuation-in-part (see Page 5)
- [X] **UZ** Uzbekistan
- [X] **VN** Viet Nam
- [X] **YU** Yugoslavia
- [X] **ZA** South Africa
- [X] **ZW** Zimbabwe

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

- [X] Mozambique
- [ ] ....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLA[redacted] ☐ Further priority [redacted]s are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 03.03.00 (3 March 2000)	09/518,914	US		
item (2) 03.05.99 (3 May 1999)	09/303,593	US		
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): 1-2

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*:

ISA / US

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following number of sheets:

request	:	6
description (excluding sequence listing part)	:	131
claims	:	27
abstract	:	1
drawings	:	35
sequence listing part of description	:	20
Total number of sheets	:	220

This international application is accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☒ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: Statement of Compliance Under 37 CFR §1.821(f) Transmittal Letter, Attachment A

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

SYNAPTIC PHARMACEUTICAL CORPORATION

Kathleen P. Mullinix April 24, 2000

NAME: Kathleen P. Mullinix Date

TITLE: President, Chairman & CEO

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

Form PCT/RO/101 (last sheet) (July 1998; reprint January 1999) *See Notes to the request form*

Box No. VI PRIORITY CLA[illegible] ☐ Further priority [illegible]re indicated in the Supplemental Box.

Filing date of earlier application *(day/month/year)*	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 03.03.00 (3 March 2000)	09/518,914	US		
item (2) 03.05.99 (3 May 1999)	09/303,593	US		
item (3)				

[X] The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): 1-2

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA / US

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following number of sheets:

request	6
description (excluding sequence listing part)	131
claims	27
abstract	1
drawings	35
sequence listing part of description	20
Total number of sheets	220

This international application is accompanied by the item(s) marked below:

1. [X] fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. [X] nucleotide and/or amino acid sequence listing in computer readable form
9. [X] other *(specify)*: Statement of Compliance Under 37 CFR §1.821(f), Transmittal Letter, Attachment A

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Beth E Borowsky 4/27/00
Beth E. Borowsky Date

Kristine L. Ogozalek 4/26/00
Kristine L. Ogozalek Date

4/26/00
Parul P. Lakhlani Date

4/26/00
Nika Adham Date

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

Supplemental Box *If [illegible]upplemental Box is not used, this sheet should no[illegible]cluded in the request.*

1. If, in any of the Boxes, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

(i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;

(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;

(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;

(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;

(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;

(vi) if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;

(vii) if, in Box No. VI, the earlier application is an ARIPO application: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed.

2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.

Continuation of Box V.: U.S. Serial No. 09/518,914, filed March 3, 2000, which is a continuation-in-part of U.S. Serial No. 09/303,593, filed May 3, 1999.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/12065

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C07K 14/705; C07H 21/04; C12N 15/63, 1/21; C12P 21/02; G01N 33/53
US CL : 530/350; 536/23.5; 436/7.1, 69.1, 252.3, 254.2, 320.1, 361
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 536/23.5; 436/7.1, 69.1, 252.3, 254.2, 320.1, 361

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Commercial Sequence Databases: GenEmbl, N_Geneseq_36, Issued_Patents_NA, EST, A_Geneseq_36, Issued Patents_AA, PIR_64, SwissProt_38, STREMBL_12

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROVENCIO, I. et al. Melanopsin: An opsin in Melanophores, Brain and Eye. Proc. Ntl. Acad. Sci. USA. January 1998. Vol. 95, pages 340-345, see entire article, especially Fig. 3.	12-14
A,P	US 6,008,338 A (Fong) 28 December 1999, see entire article.	1-43, 63-74, 76-80, 82-90, 92-98, 100, 115-119, 124-137, 143-149

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 AUGUST 2000	07 SEP 2000

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	EILEEN B. O'HARA
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/12065

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-43, 63-74, 76-80, 82-90, 92-98, 100, 115-119, 124-137 and 143-149

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/12065

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-43, 63-74, 76-80, 82-90, 92-98, 100, 115-119, 124-137 and 143-149, drawn to nucleic acids of SNORF36 receptor, vectors, host cells, SNORF36 protein, and method of making protein recombinantly and a process to screen for compounds that bind to or are antagonists or agonists of SNORF36 receptor and a method for detecting SNORF36 protein with antibody.
Group II, claim(s) 44-46 and 51-55, drawn to antisense oligonucleotides.
Group III, claim(s) 47, 48, 50 and 56, drawn to antibodies.
Group IV, claims 57, 60,61 and 62, drawn to a transgenic mammal expressing DNA encoding SNORF35 receptor.
Group V, claim(s) 58, 60, 61 and 62, drawn to a transgenic mammal comprising a homologous recombination knockout of SNORF36 receptor.
Group VI, claim(s) 59 and 62, drawn to a transgenic mammal expressing antisense DNA complementary to the DNA encoding a mammalian SNORF36 receptor.
Group VII, claim(s)49, 75, 81, 91, 105, 106, 122, 123, 141, 142 and 151, drawn to a compound that binds to SNORF36 receptor which could be an antagonist.
Group VIII, claim(s) 49, 110, 111, 120, 121, 138-140 and 150, drawn to a compound that binds to SNORF36 receptor which could be an agonist.
Group IX, claim(s) 99, 113 and 114, drawn to a method of detecting SNORF36 mRNA or DNA or a method of diagnosing a predisposition to a disorder, by nucleic acid hybrization.
Group X, claim(s)101 and 102, drawn to a method of determing the physiological effects of varying levels of mammalian SNORF36 using a transgenic mammal.
Group XI, claim(s) 103, 104, 108 and 109, drawn to a method for identifying agonists or antagonists of SNORF36 using transgenic mammals.
Group XII, claims 107 and 153, drawn to a method of treating a subject by administering a SNORF36 antagonist.
Group XIII, claims 112 and 152, drawn to a method of treating a subject by administering a SNORF36 agonist.
Group XIV, claims 154-165, drawn to a process of making a composition of matter which binds to a SNORF36 receptor.

The inventions listed as Groups do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Pursuant to 37 C.F.R. § 1.475(d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first recited product, polynucleotides encoding SNORF36 receptor proteins, vectors, host cells, SNORF36 polypeptides, a method of screening for compounds that bind to SNORF36 receptor, a method of detecting SNORF36 polypeptides and a method of recombinantly producing SNORF36. Further pursuant to 37 C.F.R. § 1.475(d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.

WO 00/66630
PCT/US00/12065
JPW

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
WHITE, John, P.
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, NY 10036
ETATS-UNIS D'AMERIQUE

RECEIVED
COOPER & DUNHAM
NOV 20 2000
DOCKET CLERK

Date of mailing (day/month/year) 09 November 2000 (09.11.00)	
Applicant's or agent's file reference 59138-B-PCT	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/US00/12065	03 May 2000 (03.05.00)	03 May 1999 (03.05.99)

Applicant
SYNAPTIC PHARMACEUTICAL CORPORATION et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
AU,KP,KR,US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD, GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX, NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on 09 November 2000 (09.11.00) under No. WO 00/66630

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2) 11/3/2001 SMB

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a **demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the **19-month time limit**. = 12/3/2000 SMB

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1)) 1/3/2001 SMB

If the applicant wishes to proceed with the international application in the **national phase**, he must, within **20 months** or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No. (41-22) 740.14.35	Authorized officer J. Zahra Telephone No. (41-22) 338.83.38

JPW

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: JOHN P. WHITE
COOPER & DUNHAM LLP
1185 AVENUE OF THE AMERICAS
NEW YORK NY 10036

Received
MAY - 7 2001
DOCKET CLERK

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	04 MAY 2001
Applicant's or agent's file reference: 59138-B-PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US00/12065	03 MAY 2000	03 MAY 1999

Applicant

SYNAPTIC PHARMACEUTICAL CORPORATION

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

11/3/2001 SMB

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	EILEEN B. O'HARA
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/416 (July 1992)*

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: JOHN P. WHITE
COOPER & DUNHAM LLP
1185 AVENUE OF THE AMERICAS
NEW YORK NY 10036

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	04 MAY 2001

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
59138-B-PCT	

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US00/12065	03 MAY 2000	03 MAY 1999

Applicant
SYNAPTIC PHARMACEUTICAL CORPORATION

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	EILEEN B. O'HARA
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/416 (July 1992)*

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 59138-B-PCT	**FOR FURTHER ACTION** See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US00/12065	International filing date *(day/month/year)* 03 MAY 2000	Priority date *(day/month/year)* 03 MAY 1999
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant SYNAPTIC PHARMACEUTICAL CORPORATION		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☒ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand 01 DECEMBER 2000	Date of completion of this report 17 APRIL 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer EILEEN B. O'HARA Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/12065

I. Basis of the report

1. With regard to the elements of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-131, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of ______

[X] the claims:
pages 132-158, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of ______

[X] the drawings:
pages 1-35, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of ______

[X] the sequence listing part of the description:
pages 1-20, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of ______

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language ______ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/ or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[X] contained in the international application in printed form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/12065

III. Non-establishment f pini n with regard to n velty, inventive step and industrial applicability

1. The questions wheth r the claimed invention appears to be novel, to involve an inventive step (to be non obvi us), or to be industrially applicable have not been and will not be examined in respect f:

☐ the entire international application.

☒ claims Nos. 44-62,75,81,91,99,101-114,120-123,138-142,150-165

because:

☐ the said international application, or the said claim Nos. _ relate to the following subject matter which does not require international preliminary examination *(specify)*.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _ are so unclear that no meaningful opinion could be formed *(specify)*.

☐ the claims, or said claims Nos. _ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. (See Attached) .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to n velty, inventive step or industrial applicability; citati ns and explanations supp rting such statement

1. statement

Novelty (N)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO
Inventive Step (IS)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO
Industrial Applicability (IA)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO

2. citations and explanations (Rule 70.7)

Claims 1-43, 63-74, 76-80, 82-90, 92-98, 100, 115-119, 124-137 and 143-149 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest the nucleic acids encoding the polypeptide identified as SNORF36 receptor, the SNORF36 receptor, and methods of screening for antagonists or agonists of the receptor. The claimed invention has industrial applicability in that the nucleic acid molecules can be used to screen for related nucleic acid molecules and the encoded protein can be used in methods to screen for compounds that bind to it or to produce antibodies.

-------------------- NEW CITATIONS --------------------
NONE

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 10

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): C07K 14/705; C07H 21/04; C12N 15/63, 1/21; C12P 21/02; G01N 33/53 and US Cl.: 530/350; 536/23.5; 436/7.1, 69.1, 252.3, 254.2, 320.1, 361

III. NON-ESTABLISHMENT OF REPORT:

No international search report has been established for claim numbers 44-62,75,81,91,99,101-114,120-123,138-142,150-165.

V. 1. REASONED STATEMENTS:
The report as to Novelty was positive (YES) with respect to claims 1-43,63-74,76-80,82-90,92-98,100,115-119,124-137,143-149.
The report as to Novelty was negative (NO) with respect to claims NONE.
The report as to Inventive Step was positive (YES) with respect to claims 1-43,63-74,76-80,82-90,92-98,100,115-119,124-137,143-149.
The report as to Inventive Step was negative (NO) with respect to claims NONE.
The report as to Industrial Applicability was positive (YES) with respect to claims 1-43,63-74,76-80,82-90,92-98,100,115-119,124-137,143-149.
The report as to Industrial Applicability was negative (NO) with respect to claims NONE.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07K 14/705, C07H 21/04, C12N 15/63, 1/21, C12P 21/02, G01N 33/53 — A1

(11) International Publication Number: WO 00/66630

(43) International Publication Date: 9 November 2000 (09.11.00)

(21) International Application Number: PCT/US00/12065

(22) International Filing Date: 3 May 2000 (03.05.00)

(30) Priority Data:

09/303,593	3 May 1999 (03.05.99)	US
09/518,914	3 March 2000 (03.03.00)	US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications

US	09/303,593 (CIP)
Filed on	3 May 1999 (03.05.99)
US	09/518,914 (CIP)
Filed on	3 March 2000 (03.03.00)

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(54) Title: DNA ENCODING SNORF36a AND SNORF36b RECEPTORS

(57) Abstract

This invention provides isolated nucleic acids encoding mammalian SNORF36 receptors, purified mammalian SNORF36 receptors, vectors comprising nucleic acid encoding mammalian SNORF36 receptors, cells comprising such vectors, antibodies directed to mammalian SNORF36 receptors, nucleic acid probes useful for detecting nucleic acid encoding mammalian SNORF36 receptors, antisense oligonucleotides complementary to unique sequences of nucleic acid encoding mammalian SNORF36 receptors, transgenic, nonhuman animals which express DNA encoding normal or mutant mammalian SNORF36 receptors, methods of isolating mammalian SNORF36 receptors, methods of treating an abnormality that is linked to the activity of the mammalian SNORF36 receptors, as well as methods of determining binding of compounds to mammalian SNORF36 receptors, methods of identifying agonists and antagonists of SNORF36 receptors, and agonists and antagonists so identified.

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DNA ENCODING SNORF36a AND SNORF36b RECEPTORS

BACKGROUND OF THE INVENTION

This application is a continuation-in-part and claims priority of U.S. Serial No. 09/518,914, filed March 3, 2000, a continuation-in-part of U.S. Serial No. 09/303,593, filed May 3, 1999, the contents of which are hereby incorporated by reference into the subject application.

Throughout this application various publications are referred to by partial citations within parenthesis. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications, in their entireties, are hereby incorporated by reference into this application in order to more fully describe the state of the art to which the invention pertains.

Neuroregulators comprise a diverse group of natural products that subserve or modulate communication in the nervous system. They include, but are not limited to, neuropeptides, amino acids, biogenic amines, lipids and lipid metabolites, and other metabolic byproducts. Many of these neuroregulator substances interact with specific cell surface receptors which transduce signals from the outside to the inside of the cell. G-protein coupled receptors (GPCRs) represent a major class of cell surface receptors with which many neurotransmitters interact to mediate their effects. GPCRs are characterized by seven membrane-spanning domains and are coupled to their effectors via G-proteins linking receptor activation with intracellular biochemical sequelae such as stimulation of adenylyl cyclase.

Opsins represent one of the major families of GPCRs. These

receptors are unique compared to other GPCRs in that light is a crucial co-factor for their activation under physiological conditions. A major subclass of the opsin family is that of visual opsins such as rhodopsin and cone opsins. The visual opsins, also known as visual photopigments, are located in the eye and are involved in transducing visual information from the eye to the brain. Our understanding of opsin function has been derived primarily from the study of visual photopigments.

Rhodopsin and cone opsins are localized in retinal rod and cone photoreceptors, respectively. These photopigments respond to different wavelengths of light and thus have very distinct absorption spectra associated with different absorption maxima (λ_{max}). Even though both receptor subtypes convey visual signals to the brain in response to illumination, they have evolved to perform very distinct functions related to vision. Cone opsins are primarily responsible for color vision, also known as photopic vision, in different species. In contrast, rhodopsin, believed to have evolved from cone opsin, is mainly involved in dim light vision, also known as scotopic vision. Rhodopsin, highly enriched in rod photoreceptor membranes, has been used extensively as a model receptor to understand activation mechanism and functioning of opsins.

Rhodopsin contains the seven membrane-spanning apoprotein opsin and a retinoid-based chromophore (See reviews Hargrave and McDowell, 1992; Yarfitz and Hurley, 1994). In the ground or inactive state (i.e. in the absence of light), the chromophore, usually 11-*cis*-retinal, is covalently attached to a highly conserved lysine residue in the middle of the seventh transmembrane segment via a protonated Schiff base. All vertebrate visual opsins contain a highly conserved glutamate residue in the transmembrane helix 3 which serves

as a counterion for the protonated Schiff base. It has been postulated that 11-*cis*-retinal behaves as an inverse agonist and induces an inactive conformation of the apoprotein which, by itself, is partially active (Cohen et al., 1993; Surya et al., 1995). Upon absorbing a photon, 11-*cis*-retinal is isomerized to the agonist all-*trans*-retinal which introduces distortion in the opsin and initiates a cascade of conformational changes in the molecule. Rhodopsin is first converted to bathorhodopsin, followed by lumirhodopsin, metarhodopsin I and metarhodopsin II states in a sequential manner. Even though most of these transient conformational states are difficult to study biochemically, they can be easily distinguished on the basis of their spectroscopic properties since each state has a unique absorption maximum. Experimental evidence suggests that the formation of metarhodopsin II, a relatively stable state, involves deprotonation of the Schiff base and represents the active conformation of the apoprotein. In this state, the opsin activates the cognate G-protein and initiates the intracellular signaling cascade which ultimately results in transfer of visual information to the brain. Upon hydrolysis of the Schiff base linkage, metarhodopsin II decays into free all-*trans*-retinal and opsin. All-*trans*-retinal is transported to the neighbouring retinal pigment epithelial cells where it is converted to 11-*cis*-retinal via enzymatic reactions. 11-*cis*-retinal is transported back to retinal photoreceptors where it recombines with the opsin apoprotein to regenerate the rhodopsin molecule.

Even though all visual opsins essentially use the same activation mechanism as rhodopsin, there are some noticeable differences between vertebrate and invertebrate visual opsins (Gartner and Towner, 1995; Yarfitz and Hurley, 1994, Terakita et al., 1998; Arnheiter, 1998). Activation of vertebrate visual pigment results primarily in stimulation of G_t G-

protein (also known as transducin) leading to an increase in cGMP phosphodiesterase activity. Initiation of this signaling cascade ultimately results in closure of cation channels and hyperpolarization of the cell. In contrast, opsin visual pigments in invertebrates such as squid and fruitfly activate G_q G-protein and elevate intracellular IP_3 and Ca^{2+} levels (Wood et al., 1989; Nobes et al., 1992; Yarfitz and Hurley, 1994). Another major difference between vertebrate and invertebrate visual opsins is the stability of the active conformation of the receptor. Formation of vertebrate metarhodopsin II, the active conformation of rhodopsin, is rapidly followed by hydrolysis of the Schiff base linkage and dissociation of metarhodopsin II into free all-*trans*-retinal and opsin apoprotein. It has been suggested that the glutamate counterion in the transmembrane helix 3 aids in the hydrolysis reaction (Gartner and Towner, 1995). In contrast, invertebrate metarhodopsin represents a thermally stable state where the chromophore remains attached to the apoprotein (Kiselev and Subramaniam, 1994). This allows rapid photoisomerization of all-*trans*-retinal back to 11-*cis*-retinal within the apoprotein and rapid regeneration of rhodopsin, thus eliminating the need for retinal regenerating tissue (Provencio et al., 1998). The thermally stable metastate of invertebrate photopigment may be formed due to the absence of the glutamate counterion in transmembrane helix 3 of invertebrate visual opsins (Gartner and Towner, 1995).

Most opsins use 11-*cis*-retinal derived from carotenoids as a chromophore; however, some opsins use 3-hydroxy, 4-hydroxy or 3,4-dehydro isomers of 11-*cis*-retinal as a chromophore to accommodate the abundant availability of the substituted carotenoids (Gartner and Towner, 1995). Different opsins respond to photons with different wavelengths, a phenomenon known as spectral tuning. Even though the use of a

particular retinal derivative as a chromophore contributes to spectral specificity (Gartner and Towner, 1995), the major determinant of spectral tuning is the presence of unique amino acids surrounding the retinal-binding site (Kochendoerfer et al., 1999). For example, substitution of a highly conserved glycine in transmembrane helix 3 of rhodopsin with amino acids of increasing size results in progressive shift of λ_{max} towards the blue wavelength (Han et al., 1996). Similarly, replacement of conserved non-polar residues with hydroxyl amino acids changes the opsin from a green-absorbing molecule to a red-absorbing pigment (Chan et al., 1992).

Even though the visual opsins have been at the forefront of opsin research, scientists are now turning their attention to non-visual opsins (the opsins not involved in transducing visual information) because of their potential involvement in physiological processes such as circadian rhythm and reproduction. The existence of non-visual photopigments in nonmammalian vertebrates was first suggested by Karl von Frisch. He demonstrated that the skin of the European minnow changed color in response to light even in the absence of the eye and pineal gland, and postulated photoreceptive elements in the diencephalon (Foster et al, 1994). Further evidence supporting the presence of non-visual photopigments was obtained in blinded lampreys and ducks which responded to illumination with body movements and gonadal induction, respectively (Foster et al., 1994). Recent studies using histochemical techniques has further corroborated these physiological observations. Silver et al. (1988) immunostained the cerebrospinal fluid (CSF)-contacting neurons with anti-opsin antibody in brains of the ring dove, quail and duck. Similarly, intense immunostaining of the CSF-contacting neurons within the basal brain of the lizard, *Anolis carolinensis,* was observed with anti-cone opsin

antibody (Foster et al., 1993).

Recent molecular cloning of several non-visual opsins is in agreement with the above-mentioned studies. Pinopsin is expressed in the pineal gland of the chicken and is believed to play a role in circadian rhythm (Okano et al., 1994; Max et al., 1995). Interestingly, expression of the pinopsin gene is regulated by light (Takanaka et al., 1998). Max et al. (1998) have demonstrated light-dependent activation of transducin by pinopsin, implying that the pinopsin is a functional photoreceptor. Two other opsins identified in pinealocytes are vertebrate ancient (VA) opsin cloned from the salmon fish (Soni et al., 1997; Soni et al., 1998), and parapinopsin cloned from the channel catfish (Blackshaw and Snyder, 1997). In addition to pineal cells, VA opsin is also localized in the amacrine and horizontal cells of the salmon retina. On the other hand, expression of parapinopsin is confined to the parapineal and pineal organs of the catfish.

Several of the non-visual opsins are, in fact, expressed in the eye. Sun et al. (1997) cloned peropsin from human retina and mouse eye. This opsin is localized exclusively in microvilli of the apical membrane of retinal pigment epithelial (RPE) cells, indicating that it may function as a sensor of retinoids generated in the adjacent outer membrane of rhodopsin or cone opsins. RPE retinal G-protein-coupled receptor (RGR) is another receptor found in the RPE (Tao et al., 1998). Unlike other opsins which are believed to be present at the plasma membrane, RGR is localized intracellularly. The amino acid sequence of RGR suggests that, along with squid retinochrome, it may form a distinct subfamily of opsins (Hara-Nishimura et al., 1990).

Interestingly, it has been suggested that RGR may prefer all-*trans*-retinal, rather than 11-*cis*-retinal, as a ligand and

may be involved in the photoisomerization of the all-*trans* isomer to the 11-*cis* isomer (Hao and Fong, 1999). In such a case, its function may be the rapid regeneration of 11-*cis*-retinal in the RPE for use in the visual cycle.

One of the known photo-sensitive processes is melanosome dispersion in the dermal melanophores of *Xenopus laevis*. In accordance with this, melanopsin has been cloned from melanophores (Provencio et al., 1998). Melanopsin is expressed in the melanophores, suprachiasmatic and preoptic nuclei of the hypothalamus, iris, RPE and retina. Its expression in visual and nonvisual tissues suggests a role in visual and nonvisual photosensory phenomena. Recently, a non-visual opsin has been identified in the mammalian brain. Blackshaw and Snyder (1999) have cloned encephalopsin, which, as the name suggests, is highly expressed in various areas of the brain. It is present in the preoptic area and the paraventricular nucleus of the hypothalamus, the cerebral cortex, cerebellar Purkinje cells, striatum, thalamus and the ventral horn of the spinal cord. Interestingly, this receptor is not present in the eye.

The molecular identification of non-visual opsins has raised several questions. How are they activated? What is their physiological function? All the non-visual opsins cloned to date contain lysine in the seventh transmembrane helix, the site for retinal chromophore attachment, implying that a retinoid may be the chromophore for the non-visual opsins, similar to the visual opsins. Several groups have been successful in reconstituting non-visual opsins with retinoids and activating them with light (Okano et al., 1994; Soni et al., 1998). Retinoids can cross the blood-brain barrier, albeit at low efficiency (Pardridge et al., 1985; Franke et al., 1999). Furthermore, a transporter with high affinity for retinoids, β-Trace, has been recognized recently (Tanaka

et al., 1997). This secretory protein is present in high levels in the CSF and may transport retinoids to different regions of the brain, analogous to the plasma RBP. That retinoids are indeed present in the brain was demonstrated by Foster et al. (1993) who were able to identify retinal isomers in the *Anolis* anterior brain using HPLC analysis. If a retinal isomer is indeed a chromophore for non-visual opsins then light would be needed to photoisomerize the isomer and activate the receptor. Several reports suggest that light can reach the deep areas of the brain (Muller and Wilson, 1986; Grace et al., 1996; Blackshaw and Snyder, 1999), and a neurotransmitter release-enhancing effect of light on cortical slices has been observed (Wade et al., 1988). Therefore, the activation mechanisms of non-visual opsins may be similar to the visual opsins. However, it should be noted here that some non-visual opsins have proven resistant to functional reconstitution with retinal isomers (Provencio et al., 1998; Blackshaw and Snyder, 1999), raising the possibility that these receptors utilize a non-retinoid ligand and may not require light for activation.

What could be the function of non-visual opsins? One interesting possibility is that they may be involved in circadian rhythm. Circadian rhythm represents daily fluctuations in biological activities that are regulated by the light-dark cycles. It is composed of three components: a photoactive input, the circadian clock itself which exhibits periodicity, and the behavioral and physiological oscillations as output. In animals, in the phenomenon known as photoentrainment, exposure to light results in regulation of the circadian rhythm. However, the identity of the photoreceptive molecule mediating photoentrainment has remained a mystery. Since the photoentrainment response occurs at the wavelength of 500 nm, it has been suggested that an opsin may be mediating the response (Foster, 1998).

Freedman et al. (1999) and Lucas et al. (1999) recently demonstrated that photoentrainment was intact in mice lacking rod and cone receptors; however, removal of the eyes in these mice abolished the effect of light on circadian rhythm as well as on melatonin synthesis. These results, and ocular localization of several non-visual opsins, strongly support the role of ocular non-visual opsins in photoentraining circadian rhythm. In addition, the non-visual opsins localized in the CNS may form a component of the circadian clock itself.

If non-visual opsins are indeed involved in regulating circadian rhythm, then they represent an attractive therapeutic target for circadian rhythm-related conditions. These include sleep disorders such as jet lag. It has been suggested that a change in circadian rhythm may be an underlying cause for sleep disorders such as insomnia, Advanced Sleep Phase Syndrome and Delayed Sleep Phase Syndrome (Sedgwick, 1998; Refinetti, 1999). In addition, dissociation between biological clock and work hours may result in shift-work-related sleep disorders. Importantly, bright light therapy has been demonstrated to help in these disorders (Rosenthal et al., 1990; Lack and Wright, 1993; Campbell et al., 1995; Murphy and Campbell, 1996; Cooke et al., 1998; Refinetti, 1999). Similarly, exposure to light at appropriate time reduces the effect of jet lag on travelers (Refinetti, 1999). These observations suggest that non-visual opsins may mediate these beneficial effects of light in circadian rhythm-related disorders.

Non-visual opsins also may play a role in seasonal affective disorder (SAD). This disorder is characterized by a subpopulation of people suffering from depression during winter. Light therapy is effective in these people (Terman and Terman, 1999); especially green light is more effective than red light (Oren et al., 1991). It has been recently

hypothesized that the interaction of specialized photoreceptors with magnetic field may influence sensitivity of patients suffering from SAD to light (Partonen, 1998).

In addition, the discreet localization of various opsins in the CNS areas indicate their potential role in CNS-related physiology and disorders.

Non-visual photoreceptors are also involved in melanosome dispersion in melanophores, and thus, in change in the color of the skin in various species. In birds and mammals, non-visual 'deep brain photoreceptors' are also linked to reproductive behaviour and photoperiodic gonadal responses (Yoshikawa and Oishi, 1998).

In summary, opsins constitute an important branch of the GPCR superfamily. They behave as photosensitive elements. They are localized in the retina and in non-retinal locations including the brain. The retinal rod and cone opsins are mainly responsible for conveying visual information to the brain, while the non-visual opsins in the retina and elsewhere may be involved in regulation of melatonin synthesis and circadian rhythm, photoentrainment, SAD, skin colour change and camouflage, and reproductive behaviour.

SUMMARY OF THE INVENTION

This invention provides an isolated nucleic acid encoding a mammalian SNORF36 receptor.

This invention further provides a purified mammalian SNORF36 receptor protein.

This invention also provides a vector comprising a nucleic acid in accordance with this invention.

This invention still further provides a cell comprising a vector in accordance with this invention.

This invention additionally provides a membrane preparation isolated from a cell in accordance with this invention.

Furthermore, this invention provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within one of the two strands of the nucleic acid encoding the mammalian SNORF36 receptor contained in plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977), plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976), plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534) or plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216).

This invention further provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figures 1A-1C (SEQ ID NO: 1), (b) the

reverse complement thereof.

This invention further provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figures 3A-3C (SEQ ID NO: 3), (b) the reverse complement thereof.

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to RNA encoding a mammalian SNORF36 receptor, so as to prevent translation of such RNA.

This invention further provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to genomic DNA encoding a mammalian SNORF36 receptor, so as to prevent transcription of such genomic DNA.

This invention also provides an antibody capable of binding to a mammalian SNORF36 receptor encoded by a nucleic acid in accordance with this invention.

Moreover, this invention provides an agent capable of competitively inhibiting the binding of an antibody in accordance with this invention to a mammalian SNORF36 receptor.

This invention yet further provides a pharmaceutical composition comprising (a) an amount of an oligonucleotide in accordance with this invention capable of passing through a cell membrane and effective to reduce expression of a mammalian SNORF36 receptor and (b) a pharmaceutically acceptable carrier capable of passing through the cell membrane.

This invention also provides a pharmaceutical composition which comprises an amount of an antibody in accordance with this invention effective to block binding of a ligand to a human SNORF36 receptor and a pharmaceutically acceptable carrier.

This invention further provides a transgenic, nonhuman mammal expressing DNA encoding a mammalian SNORF36 receptor in accordance with this invention.

This invention still further provides a transgenic, nonhuman mammal comprising a homologous recombination knockout of a native mammalian SNORF36 receptor.

This invention further provides a transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a mammalian SNORF36 receptor in accordance with this invention so placed within such genome as to be transcribed into antisense mRNA which is complementary to and hybridizes with mRNA encoding the mammalian SNORF36 receptor so as to reduce translation of such mRNA and expression of such receptor.

This invention provides a process for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises contacting cells containing DNA encoding, and expressing on their cell surface, the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor.

This invention further provides a process for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises contacting a membrane

preparation from cells containing DNA encoding, and expressing on their cell surface, the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor.

This invention still further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises separately contacting cells expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF36 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF36 receptor.

This invention further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises separately contacting a membrane preparation from cells expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the

mammalian SNORF36 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF36 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF36 receptor.

This invention further provides a compound identified by one of the processes of this invention.

This invention provides a method of screening a plurality of chemical compounds not known to bind to a mammalian SNORF36 receptor to identify a compound which specifically binds to the mammalian SNORF36 receptor, which comprises (a)contacting cells transfected with, and expressing, DNA encoding the mammalian SNORF36 receptor with a compound known to bind specifically to the mammalian SNORF36 receptor; (b)contacting the cells of step (a) with the plurality of compounds not known to bind specifically to the mammalian SNORF36 receptor, under conditions permitting binding of compounds known to bind to the mammalian SNORF36 receptor; (c) determining whether the binding of the compound known to bind to the mammalian SNORF36 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (d) separately determining the binding to the mammalian SNORF36 receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF36 receptor.

This invention further provides a method of screening a plurality of chemical compounds not known to bind to a mammalian SNORF36 receptor to identify a compound which specifically binds to the mammalian SNORF36 receptor, which comprises (a) contacting a membrane preparation from cells transfected with, and expressing, DNA encoding the mammalian

SNORF36 receptor with the plurality of compounds not known to bind specifically to the mammalian SNORF36 receptor under conditions permitting binding of compounds known to bind to the mammalian SNORF36 receptor; (b) determining whether the binding of a compound known to bind to the mammalian SNORF36 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (c) separately determining the binding to the mammalian SNORF36 receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF36 receptor.

This invention also provides a method of detecting expression of a mammalian SNORF36 receptor by detecting the presence of mRNA coding for the mammalian SNORF36 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with a nucleic acid probe according to this invention under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the mammalian SNORF36 receptor by the cell.

This invention further provides a method of detecting the presence of a mammalian SNORF36 receptor on the surface of a cell which comprises contacting the cell with an antibody according to this invention under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of the mammalian SNORF36 receptor on the surface of the cell.

This invention still further provides a method of determining the physiological effects of varying levels of activity of mammalian SNORF36 receptors which comprises producing a

transgenic, nonhuman mammal in accordance with this invention whose levels of mammalian SNORF36 receptor activity are varied by use of an inducible promoter which regulates mammalian SNORF36 receptor expression.

This invention additionally provides a method of determining the physiological effects of varying levels of activity of mammalian SNORF36 receptors which comprises producing a panel of transgenic, nonhuman mammals in accordance with this invention each expressing a different amount of mammalian SNORF36 receptor.

Moreover, this invention provides a method for identifying an antagonist capable of alleviating an abnormality wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor comprising administering a compound to a transgenic, nonhuman mammal according to this invention, and determining whether the compound alleviates any physiological and/or behavioral abnormality displayed by the transgenic, nonhuman mammal as a result of overactivity of a mammalian SNORF36 receptor, the alleviation of such an abnormality identifying the compound as an antagonist.

This invention also provides an antagonist identified by the preceding method.

This invention further provides a composition, e.g. a pharmaceutical composition, comprising an antagonist according to this invention and a carrier, e.g. a pharmaceutically acceptable carrier.

This invention additionally provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition according

to this invention so as to thereby treat the abnormality.

In addition, this invention provides a method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor comprising administering a compound to a transgenic, nonhuman mammal according to this invention, and determining whether the compound alleviates any physiological and/or behavioral abnormality displayed by the transgenic, nonhuman mammal, the alleviation of such an abnormality identifying the compound as an agonist.

This invention further provides an agonist identified by the preceding method.

This invention still further provides a composition, e.g. a pharmaceutical composition, comprising an agonist according to this invention and a carrier, e.g. a pharmaceutically acceptable carrier.

Moreover, this invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition according to this invention so as to thereby treat the abnormality.

Yet further, this invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific mammalian allele which comprises: (a) obtaining DNA of subjects suffering from the disorder; (b)performing a restriction digest of the DNA with a panel of restriction enzymes; (c) electrophoretically separating the resulting DNA fragments on a sizing gel; (d) contacting the resulting gel with a nucleic acid probe capable of

specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a mammalian SNORF36 receptor and labeled with a detectable marker; (e) detecting labeled bands which have hybridized to the DNA encoding a mammalian SNORF36 receptor to create a unique band pattern specific to the DNA of subjects suffering from the disorder; (f) repeating steps (a)-(e) with DNA obtained for diagnosis from subjects not yet suffering from the disorder; and (g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step (e) with the band pattern from step (f) for subjects not yet suffering from the disorder so as to determine whether the patterns are the same or different and thereby diagnose predisposition to the disorder if the patterns are the same.

This invention also provides a method of preparing a purified mammalian SNORF36 receptor according to the invention which comprises: (a) culturing cells which express the mammalian SNORF36 receptor; (b) recovering the mammalian SNORF36 receptor from the cells; and (c) purifying the mammalian SNORF36 receptor so recovered.

This invention further provides a method of preparing the purified mammalian SNORF36 receptor according to the invention which comprises: (a) inserting a nucleic acid encoding the mammalian SNORF36 receptor into a suitable expression vector; (b) introducing the resulting vector into a suitable host cell; (c) placing the resulting host cell in suitable conditions permitting the production of the mammalian SNORF36 receptor; (d) recovering the mammalian SNORF36 receptor so produced; and optionally (e) isolating and/or purifying the mammalian SNORF36 receptor so recovered.

Furthermore, this invention provides a process for determining whether a chemical compound is a mammalian

SNORF36 receptor agonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF36 receptor with the compound under conditions permitting the activation of the mammalian SNORF36 receptor, and detecting any increase in mammalian SNORF36 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF36 receptor agonist.

This invention also provides a process for determining whether a chemical compound is a mammalian SNORF36 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF36 receptor with the compound in the presence of a known mammalian SNORF36 receptor agonist, under conditions permitting the activation of the mammalian SNORF36 receptor, and detecting any decrease in mammalian SNORF36 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF36 receptor antagonist.

This invention still further provides a composition, for example a pharmaceutical composition, which comprises an amount of a mammalian SNORF36 receptor agonist determined by a process according to this invention effective to increase activity of a mammalian SNORF36 receptor and a carrier, for example, a pharmaceutically acceptable carrier. In one embodiment, the mammalian SNORF36 receptor agonist is not previously known.

Also, this invention provides a composition, for example a pharmaceutical composition, which comprises an amount of a mammalian SNORF36 receptor antagonist determined by a process according to this invention effective to reduce activity of a mammalian SNORF36 receptor and a carrier, for example, a pharmaceutically acceptable carrier.

This invention moreover provides a process for determining

whether a chemical compound specifically binds to and activates a mammalian SNORF36 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the chemical compound under conditions suitable for activation of the mammalian SNORF36 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change, e.g. an increase, in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian SNORF36 receptor.

This invention still further provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian SNORF36 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with both the chemical compound and a second chemical compound known to activate the mammalian SNORF36 receptor, and with only the second chemical compound, under conditions suitable for activation of the mammalian SNORF36 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change, e.g. increase, in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian SNORF36 receptor.

Further, this invention provides a compound determined by a process according to the invention and a composition, for

example, a pharmaceutical composition, which comprises an amount of a mammalian SNORF36 receptor agonist determined to be such by a process according to the invention, effective to increase activity of the mammalian SNORF36 receptor and a carrier, for example, a pharmaceutically acceptable carrier.

This invention also provides a composition, for example, a pharmaceutical composition, which comprises an amount of a mammalian SNORF36 receptor antagonist determined to be such by a process according to the invention, effective to reduce activity of the mammalian SNORF36 receptor and a carrier, for example, a pharmaceutically acceptable carrier.

This invention yet further provides a method of screening a plurality of chemical compounds not known to activate a mammalian SNORF36 receptor to identify a compound which activates the mammalian SNORF36 receptor which comprises: (a)contacting cells transfected with and expressing the mammalian SNORF36 receptor with the plurality of compounds not known to activate the mammalian SNORF36 receptor, under conditions permitting activation of the mammalian SNORF36 receptor; (b) determining whether the activity of the mammalian SNORF36 receptor is increased in the presence of one or more of the compounds; and if so (c) separately determining whether the activation of the mammalian SNORF36 receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian SNORF36 receptor.

This invention provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian SNORF36 receptor to identify a compound which inhibits the activation of the mammalian SNORF36 receptor, which comprises: (a) contacting cells transfected with and expressing the mammalian SNORF36 receptor with the plurality

of compounds in the presence of a known mammalian SNORF36 receptor agonist, under conditions permitting activation of the mammalian SNORF36 receptor; (b) determining whether the extent or amount of activation of the mammalian SNORF36 receptor is reduced in the presence of one or more of the compounds, relative to the extent or amount of activation of the mammalian SNORF36 receptor in the absence of such one or more compounds; and if so (c) separately determining whether each such compound inhibits activation of the mammalian SNORF36 receptor for each compound included in the plurality of compounds, so as to thereby identify any compound included in such plurality of compounds which inhibits the activation of the mammalian SNORF36 receptor.

This invention also provides a composition, for example a pharmaceutical composition, comprising a compound identified by a method according to this invention in an amount effective to increase mammalian SNORF36 receptor activity and a carrier, for example, a pharmaceutically acceptable carrier.

This invention still further provides a composition, for example a pharmaceutical composition, comprising a compound identified by a method according to this invention in an amount effective to decrease mammalian SNORF36 receptor activity and a carrier, for example a pharmaceutically acceptable carrier.

Furthermore, this invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject a compound which is a mammalian SNORF36 receptor agonist in an amount effective to treat the abnormality.

This invention additionally provides a method of treating an

abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject a compound which is a mammalian SNORF36 receptor antagonist in an amount effective to treat the abnormality.

This invention also provides a process for making a composition of matter which specifically binds to a mammalian SNORF36 receptor which comprises identifying a chemical compound using a process in accordance with this invention and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

This invention further provides a process for preparing a composition, for example, a pharmaceutical composition which comprises admixing a carrier, for example, a pharmaceutically acceptable carrier, and a pharmaceutically effective amount of a chemical compound identified by a process of in accordance with this invention or a novel structural and functional analog or homolog thereof.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1C

Nucleotide sequence including sequence encoding a human SNORF36a receptor (SEQ ID NO: 1). Putative open reading frames including the shortest open reading frame are indicated by underlining two start (ATG) codons (at positions 10-12 and 67-69) and the stop codon (at positions 1444-1446). In addition, partial 5' and 3' untranslated sequences are shown.

Figures 2A-2B

Deduced amino acid sequence (SEQ ID NO: 2) of the human SNORF36a receptor encoded by the longest open reading frame indicated in the nucleotide sequence shown in Figures 1A-1C (SEQ ID NO: 1). The seven putative transmembrane (TM) regions are underlined.

Figures 3A-3C

Nucleotide sequence including sequence encoding a human SNORF36b receptor (SEQ ID NO: 3). Putative open reading frames including the shortest open reading frame are indicated by underlining two start (ATG) codons (at positions 10-12 and 67-69) and the stop codon (at positions 1477-1479). In addition, partial 5' and 3' untranslated sequences are shown.

Figures 4A-4C

Deduced amino acid sequence (SEQ ID NO: 4) of the human SNORF36b receptor encoded by the longest open reading frame indicated in the nucleotide sequence shown in Figures 3A-3C (SEQ ID NO: 3). The seven putative transmembrane (TM) regions are underlined.

Figure 5

Nucleotide sequence including part of the sequence encoding a rat SNORF36 receptor (SEQ ID NO: 5).

Figure 6

Deduced partial amino acid sequence (SEQ ID NO: 6) of the rat SNORF36 receptor encoded by the nucleotide sequence shown in Figure 5 (SEQ ID NO: 5). Putative transmembrane (TM) regions are underlined.

Figures 7A-7B

Nucleotide alignment of partial sequences of human SNORF36 and rat SNORF36. Vertical lines represent identical residues.

Figure 8

Amino acid alignment of partial sequences of human SNORF36 and rat SNORF36. Vertical lines represent identical residues and dots represent similar residues.

Figures 9A-9C

Nucleotide sequence including sequence encoding a rat SNORF36 receptor (SEQ ID NO: 7). Putative open reading frames including the shortest open reading frame are indicated by underlining one start (ATG) codon (at positions 25-27) and the stop codon (at positions 1447-1449). In addition, partial 5' and 3' untranslated sequences are shown.

Figures 10A-10C

Deduced amino acid sequence (SEQ ID NO: 8) of the rat SNORF36 receptor encoded by the longest open reading frame indicated in the nucleotide sequence shown in Figures 9A-9C (SEQ ID NO: 7). The seven putative transmembrane (TM) regions are underlined.

Figures 11A-11D

Nucleotide alignment of human and rat SNORF36 receptors. Vertical lines indicate conserved residues, dots represent gaps in the alignment.

Figures 12A-12B

Amino acid alignment of human and rat SNORF36 receptors. Vertical lines indicate conserved residues, dots represent gaps in the alignment.

Figures 13A and 13B

Baseline Ca^{2+} mobilization response in human SNORF36a-transfected Cos-7 cells which were either, (1) not exposed to lamp light (Figure 13A), or (2) exposed to lamp light for 90 minutes before the experiment (Figure 13B). The response was measured using Fluorometric Imaging Plate Reader (FLIPR™). Cells were exposed to laser light at 1 second interval beginning at time 0 until the end of the trace. The first fluorescence value was subtracted from all the fluorescence values. No drug was added during the duration of the trace.

Figures 14A and 14B

Representative traces demonstrating the effect of all-*trans*-retinal (10µM) on intracellular Ca^{2+} in (1) human SNORF36a-transfected (Figure 14A), and (2) empty vector-transfected Cos-7 cells (Figure 14B). The response was measured using Fluorometric Imaging Plate Reader (FLIPR™). All-*trans*-retinal was added at time indicated by the arrow. Baseline subtraction and negative control corrections were performed on the traces.

Figures 15A and 15B

Concentration-dependent effect of retinal analogues on intracellular Ca^{2+} in human SNORF36a-transfected (1) Cos-7

cells (n=3) (Figure 15A), and (2) HEK293 cells (n=1) (Figure 15B). Data are presented as mean ± SD.

Figures 16A and 16B

Concentration-dependent effect of all-*trans*-retinal on phosphoinositide hydrolysis in human SNORF36a- and empty vector-transfected Cos-7 cells either (1) exposed to lamp light (n=2) (Figure 16A), or (2) not exposed to lamp light (n=1) (Figure 16B). Data are presented as mean ± SD.

Figures 17A and 17B

Antagonism by β-ionone (10μM) of all-*trans*-retinal-induced intracellular Ca^{2+} mobilization in human SNORF36a-transfected Cos-7 cells (n=2)(Figure 17A). Antagonism by β-ionone (10μM) of 9-*cis*-retinal-induced intracellular Ca^{2+} mobilization in human SNORF36a-transfected Cos-7 cells (n=2)(Figure 17B). The response was measured using Fluorometric Imaging Plate Reader (FLIPR™). Data are presented as mean ± SD.

Figures 18A-18C

Light sensitivity of SNORF36a expressed in oocytes. Oocytes shown in Figure 18A and Figure 18B were injected with 10 ng SNORF36a synthetic mRNA. The current is expressed in nA (nanoampere) while time is expressed in s (second).

Figure 18A: Current elicited by light exposure (bar) in a voltage-clamped oocyte expressing SNORF36a. The oocyte was pre-incubated with all-*trans*-retinal (100 nM) in the dark for 24 hours.

Figure 18B: Response of a second oocyte pre-incubated with all-*trans*-retinal that had been previously exposed to room light.

Figure 18C: Control uninjected oocyte, pre-incubated with

all-*trans*-retinal (100 nM) for 24 h, fails to respond to light.

Figure 19

Summary of light sensitivity of oocytes expressing SNORF36 pre-incubated with all-*trans*-retinal, 13-*cis*-retinal and ATRA (all-*trans* retinoic acid). Numbers in parentheses are the numbers of oocytes used.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a recombinant nucleic acid comprising a nucleic acid encoding a mammalian SNORF36a receptor, wherein the mammalian receptor-encoding nucleic acid hybridizes under high stringency conditions to a nucleic acid encoding a human SNORF36a receptor and having a sequence identical to the sequence of the human SNORF36a receptor-encoding nucleic acid contained in plasmid pcDNA3.1-hSNORF36a-f (ATCC Patent Depository No. 203977).

This invention further provides a recombinant nucleic acid comprising a nucleic acid encoding a human SNORF36a receptor, wherein the human SNORF36a receptor comprises an amino acid sequence identical to the sequence of the human SNORF36a receptor encoded by the shortest open reading frame indicated in Figures 1A-1C (SEQ ID NO: 1).

This invention provides a recombinant nucleic acid comprising a nucleic acid encoding a mammalian SNORF36b receptor, wherein the mammalian receptor-encoding nucleic acid hybridizes under high stringency conditions to a nucleic acid encoding a human SNORF36b receptor and having a sequence identical to the sequence of the human SNORF36b receptor-encoding nucleic acid contained in plasmid pcDNA3.1-hSNORF36b-f (ATCC Patent Depository No. 203976).

This invention also provides a recombinant nucleic acid comprising a nucleic acid encoding a human SNORF36b receptor, wherein the human SNORF36b receptor comprises an amino acid sequence identical to the sequence of the human SNORF36b receptor encoded by the shortest open reading frame indicated in Figures 3A-3C (SEQ ID NO: 3).

This invention also contemplates recombinant nucleic acids

which comprise nucleic acids encoding naturally occurring allelic variants of the above. For example, one such allelic variant involves changing Guanine (G) to Adenine (A) at position 39 in Figures 1A-1C (SEQ ID NO: 1). Another example of such an allelic variant involves changing Guanine (G) to Adenine (A) at position 39 in Figures 3A-3C (SEQ ID NO: 3).

The plasmid pcDNA3.1-hSNORF36a-f and plasmid pcDNA3.1-hSNORF36b-f were both deposited on April 28, 1999, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and were accorded ATCC Patent Depository Nos. 203977 and 203976, respectively.

The plasmid pEXJ.T3T7-rSNORF36p was deposited on August 17, 1999, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and were accorded ATCC Patent Depository No. PTA-534.

The plasmid pEXJ.T7-rSNORF36-f was deposited on January 18, 2000, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and were accorded ATCC Patent Depository No. PTA-1216.

Hybridization methods are well known to those of skill in the art. For purposes of this invention, hybridization under high stringency conditions means hybridization performed at 40°C in a hybridization buffer containing 50% formamide, 5X SSC,

7mM Tris, 1X Denhardt's, 25μg/ml salmon sperm DNA; wash at 50°C in 0.1X SSC, 0.1%SDS.

Throughout this application, the following standard abbreviations are used to indicate specific nucleotide bases:

A = adenine
G = guanine
C = cytosine
T = thymine
M = adenine or cytosine
R = adenine or guanine
W = adenine or thymine
S = cytosine or guanine
Y = cytosine or thymine
K = guanine or thymine
V = adenine, cytosine, or guanine (not thymine)
H = adenine, cytosine, or thymine (not cytosine)
B = cytosine, guanine, or thymine (not adenine)
N = adenine, cytosine, guanine, or thymine (or other modified base such as inosine)
I = inosine

Furthermore, the term "agonist" is used throughout this application to indicate any peptide or non-peptidyl compound which increases the activity of any of the polypeptides of the subject invention. The term "antagonist" is used throughout this application to indicate any peptide or non-peptidyl compound which decreases the activity of any of the polypeptides of the subject invention.

Furthermore, as used herein, the phrase "pharmaceutically acceptable carrier" means any of the standard pharmaceutically acceptable carriers. Examples include, but are not limited to, phosphate buffered saline, physiological saline, water, and emulsions, such as oil/water emulsions.

It is possible that the mammalian SNORF36 receptor gene contains introns and furthermore, the possibility exists that additional introns could exist in coding or non-coding regions. In addition, spliced form(s) of mRNA may encode additional amino acids either upstream of the currently defined starting methionine or within the coding region. Further, the existence and use of alternative exons is possible, whereby the mRNA may encode different amino acids within the region comprising the exon. In addition, single amino acid substitutions may arise via the mechanism of RNA editing such that the amino acid sequence of the expressed protein is different than that encoded by the original gene. (Burns, et al., 1996; Chu, et al., 1996). Such variants may exhibit pharmacologic properties differing from the polypeptide encoded by the original gene.

This invention provides splice variants of the mammalian SNORF36 receptors disclosed herein. This invention further provides for alternate translation initiation sites and alternately spliced or edited variants of nucleic acids encoding the SNORF36 receptors of this invention.

This invention also contemplates recombinant nucleic acids which comprise nucleic acids encoding naturally occurring allelic variants of the SNORF36 receptors disclosed herein.

The nucleic acids of the subject invention also include nucleic acid analogs of the human SNORF36a receptor genes, wherein the human SNORF36a receptor gene comprises the nucleic acid sequence shown in Figures 1A-1C (SEQ ID NO: 1) or contained in plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). Nucleic acid analogs of the human SNORF36a receptor genes differ from the human SNORF36a receptor genes described herein in terms of the identity or location of one or more nucleic acid bases (deletion analogs containing less than all of the nucleic acid bases shown in Figures 1A-1C or

contained in plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977), substitution analogs wherein one or more nucleic acid bases shown in Figures 1A-1C or contained in plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977), are replaced by other nucleic acid bases, and addition analogs, wherein one or more nucleic acid bases are added to a terminal or medial portion of the nucleic acid sequence) and which encode proteins which share some or all of the properties of the proteins encoded by the nucleic acid sequences shown in Figures 1A-1C or contained in plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). In one embodiment of the present invention, the nucleic acid analog encodes a protein which has an amino acid sequence identical to that shown in Figures 2A-2B or encoded by the nucleic acid sequence contained in pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). In another embodiment, the nucleic acid analog encodes a protein having an amino acid sequence which differs from the amino acid sequences shown in Figures 2A-2B or encoded by the nucleic acid contained in plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). In a further embodiment, the protein encoded by the nucleic acid analog has a function which is the same as the function of the receptor proteins having the amino acid sequence shown in Figures 2A-2B. In another embodiment, the function of the protein encoded by the nucleic acid analog differs from the function of the receptor protein having the amino acid sequence shown in Figures 2A-2B. In another embodiment, the variation in the nucleic acid sequence occurs within the transmembrane (TM) region of the protein. In a further embodiment, the variation in the nucleic acid sequence occurs outside of the TM region.

The nucleic acids of the subject invention also include nucleic acid analogs of the human SNORF36b receptor genes, wherein the human SNORF36b receptor gene comprises the nucleic acid sequence shown in Figures 3A-3C (SEQ ID NO: 3)

or contained in plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). Nucleic acid analogs of the human SNORF36b receptor genes differ from the human SNORF36b receptor genes described herein in terms of the identity or location of one or more nucleic acid bases (deletion analogs containing less than all of the nucleic acid bases shown in Figures 3A-3C or contained in plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976), substitution analogs wherein one or more nucleic acid bases shown in Figures 3A-3C or contained in plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976), are replaced by other nucleic acid bases, and addition analogs, wherein one or more nucleic acid bases are added to a terminal or medial portion of the nucleic acid sequence) and which encode proteins which share some or all of the properties of the proteins encoded by the nucleic acid sequences shown in Figures 3A-3C or contained in plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). In one embodiment of the present invention, the nucleic acid analog encodes a protein which has an amino acid sequence identical to that shown in Figures 4A-4C or encoded by the nucleic acid sequence contained in pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). In another embodiment, the nucleic acid analog encodes a protein having an amino acid sequence which differs from the amino acid sequences shown in Figures 4A-4C or encoded by the nucleic acid contained in plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). In a further embodiment, the protein encoded by the nucleic acid analog has a function which is the same as the function of the receptor proteins having the amino acid sequence shown in Figures 4A-4C. In another embodiment, the function of the protein encoded by the nucleic acid analog differs from the function of the receptor protein having the amino acid sequence shown in Figures 4A-4C. In another embodiment, the variation in the nucleic acid sequence occurs within the transmembrane (TM) region of the protein. In a further embodiment, the variation in the nucleic acid sequence occurs

outside of the TM region.

The nucleic acids of the subject invention also include nucleic acid analogs of the rat SNORF36 receptor genes, wherein the rat SNORF36 receptor gene comprises the nucleic acid sequence shown in Figure 5 or contained in plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534). Nucleic acid analogs of the rat SNORF36 receptor genes differ from the rat SNORF36 receptor genes described herein in terms of the identity or location of one or more nucleic acid bases deletion analogs containing less than all of the nucleic acid bases shown in Figure 5 or contained in plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534), substitution analogs wherein one or more nucleic acid bases shown in Figure 5 or contained in plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534), are replaced by other nucleic acid bases, and addition analogs, wherein one or more nucleic acid bases are added to a terminal or medial portion of the nucleic acid sequence) and which encode proteins which share some or all of the properties of the proteins encoded by the nucleic acid sequences shown in Figure 5 or contained in plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534). In one embodiment of the present invention, the nucleic acid analog encodes a protein which has an amino acid sequence identical to that shown in Figure 6 or encoded by the nucleic acid sequence contained in plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534). In another embodiment, the nucleic acid analog encodes a protein having an amino acid sequence which differs from the amino acid sequences shown in Figure 6 or encoded by the nucleic acid contained in plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534). In a further embodiment, the protein encoded by the nucleic acid analog has a function which is the same as the function of the receptor proteins having the amino acid sequence shown in Figure 6. In another embodiment, the function of the protein encoded by the

nucleic acid analog differs from the function of the receptor protein having the amino acid sequence shown in Figure 6. In another embodiment, the variation in the nucleic acid sequence occurs within the transmembrane (TM) region of the protein. In a further embodiment, the variation in the nucleic acid sequence occurs outside of the TM region.

The nucleic acids of the subject invention also include nucleic acid analogs of the rat SNORF36 receptor genes, wherein the rat SNORF36 receptor gene comprises the nucleic acid sequence shown in Figures 9A-9C or contained in plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). Nucleic acid analogs of the rat SNORF36 receptor genes differ from the rat SNORF36 receptor genes described herein in terms of the identity or location of one or more nucleic acid bases deletion analogs containing less than all of the nucleic acid bases shown in Figures 9A-9C or contained in plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216), substitution analogs wherein one or more nucleic acid bases shown in Figures 9A-9C or contained in plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216), are replaced by other nucleic acid bases, and addition analogs, wherein one or more nucleic acid bases are added to a terminal or medial portion of the nucleic acid sequence) and which encode proteins which share some or all of the properties of the proteins encoded by the nucleic acid sequences shown in Figures 9A-9C or contained in plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). In one embodiment of the present invention, the nucleic acid analog encodes a protein which has an amino acid sequence identical to that shown in Figures 10A-10C or encoded by the nucleic acid sequence contained in plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). In another embodiment, the nucleic acid analog encodes a protein having an amino acid sequence which differs from the amino acid sequences shown in Figures 10A-10C or encoded by the nucleic

acid contained in plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). In a further embodiment, the protein encoded by the nucleic acid analog has a function which is the same as the function of the receptor proteins having the amino acid sequence shown in Figures 10A-10C. In another embodiment, the function of the protein encoded by the nucleic acid analog differs from the function of the receptor protein having the amino acid sequence shown in Figures 10A-10C. In another embodiment, the variation in the nucleic acid sequence occurs within the transmembrane (TM) region of the protein. In a further embodiment, the variation in the nucleic acid sequence occurs outside of the TM region.

This invention provides the above-described isolated nucleic acid, wherein the nucleic acid is DNA. In an embodiment, the DNA is cDNA. In another embodiment, the DNA is genomic DNA. In still another embodiment, the nucleic acid is RNA. Methods for production and manipulation of nucleic acid molecules are well known in the art.

This invention further provides nucleic acid which is degenerate with respect to the DNA encoding any of the polypeptides described herein. In an embodiment, the nucleic acid comprises a nucleotide sequence which is degenerate with respect to the nucleotide sequence shown in Figures 1A-1C (SEQ ID NO: 1) or the nucleotide sequence contained in the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977), that is, a nucleotide sequence which is translated into the same amino acid sequence. In another embodiment, the nucleic acid comprises a nucleotide sequence which is degenerate with respect to the nucleotide sequence shown in Figures 3A-3C (SEQ ID NO: 3) or the nucleotide sequence contained in the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976), that is, a nucleotide sequence which is translated into the same amino acid sequence.

In another embodiment, the nucleic acid comprises a nucleotide sequence which is degenerate with respect to the nucleotide sequence shown in Figure 5 (SEQ ID NO: 5) or Figures 9A-9C (SEQ ID NO: 7) or the nucleotide sequence contained in the plasmids pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534) or pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216), that is, a nucleotide sequence which is translated into the same amino acid sequence.

This invention also encompasses DNAs and cDNAs which encode amino acid sequences which differ from those of the polypeptides of this invention, but which should not produce phenotypic changes.

Alternately, this invention also encompasses DNAs, cDNAs, and RNAs which hybridize to the DNA, cDNA, and RNA of the subject invention. Hybridization methods are well known to those of skill in the art.

The nucleic acids of the subject invention also include nucleic acid molecules coding for polypeptide analogs, fragments or derivatives of antigenic polypeptides which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (deletion analogs containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues specified are replaced by other residues and addition analogs wherein one or more amino acid residues is added to a terminal or medial portion of the polypeptides) and which share some or all properties of naturally-occurring forms. These molecules include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily expressed vectors. The

creation of polypeptide analogs is well known to those of skill in the art (Spurney, R. F. et al. (1997); Fong, T.M. et al. (1995); Underwood, D.J. et al. (1994); Graziano, M.P. et al. (1996); Guan X.M. et al. (1995)).

The modified polypeptides of this invention may be transfected into cells either transiently or stably using methods well-known in the art, examples of which are disclosed herein. This invention also provides for binding assays using the modified polypeptides, in which the polypeptide is expressed either transiently or in stable cell lines. This invention further provides a compound identified using a modified polypeptide in a binding assay such as the binding assays described herein.

The nucleic acids described and claimed herein are useful for the information which they provide concerning the amino acid sequence of the polypeptide and as products for the large scale synthesis of the polypeptides by a variety of recombinant techniques. The nucleic acid molecule is useful for generating new cloning and expression vectors, transformed and transfected prokaryotic and eukaryotic host cells, and new and useful methods for cultured growth of such host cells capable of expression of the polypeptide and related products.

This invention also provides an isolated nucleic acid encoding species homologs of the SNORF36 receptor encoded by the nucleic acid sequence shown in Figures 1A-1C (SEQ ID NO: 1) or encoded by the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). In one embodiment, the nucleic acid encodes a mammalian SNORF36 receptor homolog which has substantially the same amino acid sequence as does the SNORF36 receptor encoded by the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). In another embodiment, the nucleic acid encodes a mammalian SNORF36 receptor homolog

which has above 75% amino acid identity to the SNORF36 receptor encoded by the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977); preferably above 85% amino acid identity to the SNORF36 receptor encoded by the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977); most preferably above 95% amino acid identity to the SNORF36 receptor encoded by the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). In another embodiment, the mammalian SNORF36 receptor homolog has above 70% nucleic acid identity to the SNORF36 receptor gene contained in plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977); preferably above 80% nucleic acid identity to the SNORF36 receptor gene contained in the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977); more preferably above 90% nucleic acid identity to the SNORF36 receptor gene contained in the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). Examples of methods for isolating and purifying species homologs are described elsewhere (e.g., U.S. Patent No. 5,602,024, WO94/14957, WO97/26853, WO98/15570).

This invention also provides an isolated nucleic acid encoding species homologs of the SNORF36 receptors encoded by the nucleic acid sequence shown in Figures 3A-3C (SEQ ID NO: 3) or encoded by the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). In one embodiment, the nucleic acid encodes a mammalian SNORF36 receptor homolog which has substantially the same amino acid sequence as does the SNORF36 receptor encoded by the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). In another embodiment, the nucleic acid encodes a mammalian SNORF36 receptor homolog which has above 75% amino acid identity to the SNORF36 receptor encoded by the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976); preferably above 85% amino acid identity to the SNORF36 receptor encoded by the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976); most preferably above 95% amino acid identity to the SNORF36

receptor encoded by the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). In another embodiment, the mammalian SNORF36 receptor homolog has above 70% nucleic acid identity to the SNORF36 receptor gene contained in plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976); preferably above 80% nucleic acid identity to the SNORF36 receptor gene contained in the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976); more preferably above 90% nucleic acid identity to the SNORF36 receptor gene contained in the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976).

This invention also provides an isolated nucleic acid encoding species homologs of the SNORF36 receptors encoded by the nucleic acid sequence shown in Figure 5 (SEQ ID NO: 5) or encoded by the plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534). In one embodiment, the nucleic acid encodes a mammalian SNORF36 receptor homolog which has substantially the same amino acid sequence as does the SNORF36 receptor encoded by the plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534). In another embodiment, the nucleic acid encodes a mammalian SNORF36 receptor homolog which has above 75% amino acid identity to the SNORF36 receptor encoded by the plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534); preferably above 85% amino acid identity to the SNORF36 receptor encoded by the plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534); most preferably above 95% amino acid identity to the SNORF36 receptor encoded by the plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534). In another embodiment, the mammalian SNORF36 receptor homolog has above 70% nucleic acid identity to the SNORF36 receptor gene contained in plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534); preferably above 80% nucleic acid identity to the SNORF36 receptor gene contained in the plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534); more preferably above 90% nucleic acid identity to the SNORF36 receptor gene

contained in the plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534).

This invention also provides an isolated nucleic acid encoding species homologs of the SNORF36 receptors encoded by the nucleic acid sequence shown in Figures 9A-9C (SEQ ID NO: 7) or encoded by the plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). In one embodiment, the nucleic acid encodes a mammalian SNORF36 receptor homolog which has substantially the same amino acid sequence as does the SNORF36 receptor encoded by the plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). In another embodiment, the nucleic acid encodes a mammalian SNORF36 receptor homolog which has above 75% amino acid identity to the SNORF36 receptor encoded by the pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216); preferably above 85% amino acid identity to the SNORF36 receptor encoded by the plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216); most preferably above 95% amino acid identity to the SNORF36 receptor encoded by the plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). In another embodiment, the mammalian SNORF36 receptor homolog has above 70% nucleic acid identity to the SNORF36 receptor gene contained in plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216); preferably above 80% nucleic acid identity to the SNORF36 receptor gene contained in the plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216); more preferably above 90% nucleic acid identity to the SNORF36 receptor gene contained in the plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216).

This invention provides an isolated nucleic acid encoding a modified mammalian SNORF36 receptor, which differs from a mammalian SNORF36 receptor by having an amino acid(s) deletion, replacement, or addition in the third intracellular domain.

This invention provides an isolated nucleic acid encoding a mammalian SNORF36 receptor. In one embodiment, the nucleic acid is DNA. In another embodiment, the DNA is cDNA. In another embodiment, the DNA is genomic DNA. In another embodiment, the nucleic acid is RNA.

In another embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor. In another embodiment, the human SNORF36a receptor has an amino acid sequence identical to that encoded by the pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). In another embodiment, the human SNORF36a receptor has an amino acid sequence identical to the amino acid sequence shown in Figures 2A-2B (SEQ ID NO: 2).

In another embodiment, the mammalian SNORF36 receptor is a human SNORF36b receptor. In another embodiment, the human SNORF36b receptor has an amino acid sequence identical to that encoded by the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). In another embodiment, the human SNORF36b receptor has an amino acid sequence identical to the amino acid sequence shown in Figures 4A-4C (SEQ ID NO: 4).

In an embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor. In another embodiment, the rat SNORF36 receptor has an amino acid sequence identical to that encoded by the plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). In another embodiment, the rat SNORF36 receptor has an amino acid sequence identical to the amino acid sequence shown in Figures 10A-10C (SEQ ID NO: 8).

This invention provides a purified mammalian SNORF36 receptor protein. In one embodiment, the SNORF36 receptor protein is a human SNORF36a receptor protein. In another embodiment, the SNORF36 receptor protein is a human SNORF36b receptor protein. In a further embodiment, the SNORF36 receptor protein is a rat SNORF36 receptor protein.

This invention provides a vector comprising the nucleic acid of this invention. This invention further provides a vector adapted for expression in a cell which comprises the regulatory elements necessary for expression of the nucleic acid in the cell operatively linked to the nucleic acid encoding the receptor so as to permit expression thereof, wherein the cell is a bacterial, amphibian, yeast, insect or mammalian cell. In one embodiment, the vector is a baculovirus. In another embodiment, the vector is a plasmid.

This invention provides a plasmid designated pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). This invention also provides a plasmid designated pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). This invention provides a plasmid designated pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534). This invention provides a plasmid designated pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216).

This invention further provides for any vector or plasmid which comprises modified untranslated sequences, which are beneficial for expression in desired host cells or for use in binding or functional assays. For example, a vector or plasmid with untranslated sequences of varying lengths may express differing amounts of the polypeptide depending upon the host cell used. In an embodiment, the vector or plasmid comprises the coding sequence of the polypeptide and the regulatory elements necessary for expression in the host cell.

This invention provides for a cell comprising the vector of this invention. In one embodiment, the cell is a non-mammalian cell. In one embodiment, the non-mammalian cell is a *Xenopus* oocyte cell or a *Xenopus* melanophore cell. In another embodiment, the cell is a mammalian cell. In another embodiment, the cell is a COS-7 cell, a 293 human embryonic

kidney cell, a NIH-3T3 cell, a LM(tk-) cell, a mouse Y1 cell, or a CHO cell. In another embodiment, the cell is an insect cell. In another embodiment, the insect cell is an Sf9 cell, an Sf21 cell or a Trichoplusia ni 5B-4 cell.

This invention provides a membrane preparation isolated from the cell in accordance with this invention.

Furthermore, this invention provides for a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within one of the two strands of the nucleic acid encoding the mammalian SNORF36 receptor contained in plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977), plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976), plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534) or plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216).

This invention further provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figures 1A-1C (SEQ ID NO: 1) or (b) the reverse complement thereof. This invention further provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figures 3A-3C (SEQ ID NO: 3) or (b) the reverse complement thereof. This invention also provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein

the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figure 5 (SEQ ID NO: 5) or (b) the reverse complement thereof. This invention also provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figures 9A-9C (SEQ ID NO: 7) or (b) the reverse complement thereof. In one embodiment, the nucleic acid is DNA. In another embodiment, the nucleic acid is RNA.

As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs.

The nucleic acids of this invention may be used as probes to obtain homologous nucleic acids from other species and to detect the existence of nucleic acids having complementary sequences in samples.

The nucleic acids may also be used to express the receptors they encode in transfected cells.

The use of a constitutively active receptor encoded by SNORF36 either occurring naturally without further modification or after appropriate point mutations, deletions or the like, allows screening for antagonists and *in vivo* use of such antagonists to attribute a role to receptor SNORF36 without prior knowledge of the endogenous ligand.

Use of the nucleic acids further enables elucidation of possible receptor diversity and of the existence of multiple

subtypes within a family of receptors of which SNORF36 is a member.

Finally, it is contemplated that this receptor will serve as a valuable tool for designing drugs for treating various pathophysiological conditions such as chronic and acute inflammation, arthritis, autoimmune diseases, transplant rejection, graft vs. host disease, bacterial, fungal, protozoan and viral infections, septicemia, AIDS, pain, psychotic and neurological disorders, including anxiety, depression, schizophrenia, dementia, mental retardation, memory loss, epilepsy, neuromotor disorders, locomotor problems, respiratory disorders, asthma, eating/body weight disorders including obesity, bulimia, diabetes, anorexia, nausea, hypertension, hypotension, vascular and cardiovascular disorders, ischemia, stroke, cancers, ulcers, urinary retention, sexual/reproductive disorders, circadian rhythm disorders, renal disorders, bone diseases including osteoporosis, benign prostatic hypertrophy, gastrointestinal disorders, nasal congestion, dermatological disorders such as psoriasis, allergies, Parkinson's disease, Alzheimer's disease, acute heart failure, angina disorders, delirium, dyskinesias such as Huntington's disease or Gille's de la Tourette's syndrome, among others and diagnostic assays for such conditions. This receptor may also serve as a valuable tool for designing drugs for chemoprevention.

Methods of transfecting cells e.g. mammalian cells, with such nucleic acid to obtain cells in which the receptor is expressed on the surface of the cell are well known in the art. (See, for example, U.S. Patent Nos. 5,053,337; 5,155,218; 5,360,735; 5,472,866; 5,476,782; 5,516,653; 5,545,549; 5,556,753; 5,595,880; 5,602,024; 5,639,652; 5,652,113; 5,661,024; 5,766,879; 5,786,155; and 5,786,157, the disclosures of which are hereby incorporated by reference in their entireties into this application.)

Such transfected cells may also be used to test compounds and screen compound libraries to obtain compounds which bind to the SNORF36 receptor, as well as compounds which activate or inhibit activation of functional responses in such cells, and therefore are likely to do so in vivo. (See, for example, U.S. Patent Nos. 5,053,337; 5,155,218; 5,360,735; 5,472,866; 5,476,782; 5,516,653; 5,545,549; 5,556,753; 5,595,880; 5,602,024; 5,639,652; 5,652,113; 5,661,024; 5,766,879; 5,786,155; and 5,786,157, the disclosures of which are hereby incorporated by reference in their entireties into this application.)

This invention further provides an antibody capable of binding to a mammalian SNORF36 receptor encoded by a nucleic acid encoding a mammalian receptor. In one embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In a further embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor.

This invention also provides an agent capable of competitively inhibiting the binding of the antibody to a mammalian SNORF36 receptor. In one embodiment, the antibody is a monoclonal antibody or antisera.

Methods of preparing and employing antisense oligonucleotides, antibodies, nucleic acid probes and transgenic animals directed to the SNORF36 receptor are well known in the art. (See, for example, U.S. Patent Nos. 5,053,337; 5,155,218; 5,360,735; 5,472,866; 5,476,782; 5,516,653; 5,545,549; 5,556,753; 5,595,880; 5,602,024; 5,639,652; 5,652,113; 5,661,024; 5,766,879; 5,786,155; and 5,786,157, the disclosures of which are hereby incorporated by reference in their entireties into this application.)

This invention provides for an antisense oligonucleotide having a sequence capable of specifically hybridizing to RNA

encoding a mammalian SNORF36 receptor, so as to prevent translation of such RNA. This invention further provides for an antisense oligonucleotide having a sequence capable of specifically hybridizing to genomic DNA encoding a mammalian SNORF36 receptor, so as to prevent transcription of such genomic DNA. In one embodiment, the oligonucleotide comprises chemically modified nucleotides or nucleotide analogues.

This invention also provides for an antibody capable of binding to a mammalian SNORF36 receptor encoded by a nucleic acid in accordance with this invention. In an embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In a further embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor.

Moreover, this invention provides an agent capable of competitively inhibiting the binding of an antibody in accordance with this invention to a mammalian SNORF36 receptor. In one embodiment, the antibody is a monoclonal antibody or antisera.

This invention still further provides a pharmaceutical composition comprising (a) an amount of an oligonucleotide in accordance with this invention capable of passing through a cell membrane and effective to reduce expression of a mammalian SNORF36 receptor and (b) a pharmaceutically acceptable carrier capable of passing through the cell membrane.

In one embodiment, the oligonucleotide is coupled to a substance which inactivates mRNA. In another embodiment, the substance which inactivates mRNA is a ribozyme. In another embodiment, the pharmaceutically acceptable carrier comprises a structure which binds to a mammalian SNORF36 receptor on a cell capable of being taken up by the cells after binding

to the structure. In another embodiment, the pharmaceutically acceptable carrier is capable of binding to a mammalian SNORF36 receptor which is specific for a selected cell type.

This invention also provides a pharmaceutical composition which comprises an amount of an antibody in accordance with this invention effective to block binding of a ligand to a human SNORF36a receptor or a human SNORF36b receptor and a pharmaceutically acceptable carrier.

This invention further provides a transgenic, nonhuman mammal expressing DNA encoding a mammalian SNORF36 receptor in accordance with this invention. This invention provides a transgenic, nonhuman mammal comprising a homologous recombination knockout of a native mammalian SNORF36 receptor. This invention further provides a transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a mammalian SNORF36 receptor in accordance with this invention so placed within such genome as to be transcribed into antisense mRNA which is complementary and hybridizes with mRNA encoding the mammalian SNORF36 receptor so as to thereby reduce translation or such mRNA and expression of such receptor. In one embodiment, the DNA encoding the mammalian SNORF36 receptor additionally comprises an inducible promoter. In another embodiment, the DNA encoding the mammalian SNORF36 receptor additionally comprises tissue specific regulatory elements. In another embodiment, the transgenic, nonhuman mammal is a mouse.

This invention provides for a process for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises contacting cells containing DNA encoding, and expressing on their cell surface, the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the

compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor. This invention further provides for a process for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises contacting a membrane preparation from cells containing DNA encoding and expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor.

In an embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In another embodiment, the mammalian SNORF36 receptor has substantially the same amino acid sequence as the human SNORF36a receptor encoded by plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). In another embodiment, the mammalian SNORF36 receptor has substantially the same amino acid sequence as the human SNORF36b receptor encoded by plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). In another embodiment, the mammalian SNORF36 receptor has substantially the same amino acid sequence as that shown in Figures 2A-2B (SEQ ID NO: 2) or Figures 4A-4C (SEQ ID NO: 4). In another embodiment, the mammalian SNORF36 receptor has the amino acid sequence shown in Figures 2A-2B (SEQ ID NO: 2) or Figures 4A-4C (SEQ ID NO: 4).

In another embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor. In another embodiment, the mammalian SNORF36 receptor has substantially the same amino acid sequence as the rat SNORF36 receptor encoded by plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). In another embodiment, the mammalian SNORF36 receptor has substantially the same amino acid sequence as that shown in

Figures 10A-10C (SEQ ID NO: 8). In another embodiment, the mammalian SNORF36 receptor has the amino acid sequence shown in Figures 10A-10C (SEQ ID NO: 8).

In one embodiment, the compound is not previously known to bind to a mammalian SNORF36 receptor. In one embodiment, the cell is an insect cell. In one embodiment, the cell is a mammalian cell. In another embodiment, the cell is nonneuronal in origin. In another embodiment, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell. In another embodiment, the compound is a compound not previously known to bind to a mammalian SNORF36 receptor. This invention provides a compound identified by the preceding process according to this invention.

This invention still further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises separately contacting cells expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF36 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF36 receptor.

This invention provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which

comprises separately contacting a membrane preparation from cells expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF36 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF36 receptor.

In an embodiment of the present invention, the second chemical compound is a retinoic acid derivative. Examples of retinoic acid derivatives include, but are not limited to, all-*trans* retinoic acid (ATRA), 9-*cis*-retinal, 13-*cis*-retinal, and all-*trans*-retinal.

In one embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In another embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor. In a further embodiment, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In another embodiment, the cell is nonneuronal in origin. In another embodiment, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell. In another embodiment, the compound is not previously known to bind to a mammalian SNORF36 receptor. This invention provides for a compound identified by the preceding process according to this invention.

This invention provides for a method of screening a plurality

of chemical compounds not known to bind to a mammalian SNORF36 receptor to identify a compound which specifically binds to the mammalian SNORF36 receptor, which comprises (a) contacting cells transfected with, and expressing, DNA encoding the mammalian SNORF36 receptor with a compound known to bind specifically to the mammalian SNORF36 receptor; (b) contacting the cells of step (a) with the plurality of compounds not known to bind specifically to the mammalian SNORF36 receptor, under conditions permitting binding of compounds known to bind to the mammalian SNORF36 receptor; (c) determining whether the binding of the compound known to bind to the mammalian SNORF36 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (d) separately determining the binding to the mammalian SNORF36 receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF36 receptor.

This invention provides a method of screening a plurality of chemical compounds not known to bind to a mammalian SNORF36 receptor to identify a compound which specifically binds to the mammalian SNORF36 receptor, which comprises (a) contacting a membrane preparation from cells transfected with, and expressing, DNA encoding the mammalian SNORF36 receptor with the plurality of compounds not known to bind specifically to the mammalian SNORF36 receptor under conditions permitting binding of compounds known to bind to the mammalian SNORF36 receptor; (b) determining whether the binding of a compound known to bind to the mammalian SNORF36 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (c) separately determining the binding to the mammalian SNORF36 receptor of each compound included in the plurality of

compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF36 receptor.

In one embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In a further embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor. In another embodiment, the cell is a mammalian cell. In another embodiment, the mammalian cell is non-neuronal in origin. In a further embodiment, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell, a CHO cell, a mouse Y1 cell, or an NIH-3T3 cell.

This invention also provides a method of detecting expression of a mammalian SNORF36 receptor by detecting the presence of mRNA coding for the mammalian SNORF36 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with a nucleic acid probe according to this invention under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the mammalian SNORF36 receptor by the cell.

This invention further provides for a method of detecting the presence of a mammalian SNORF36 receptor on the surface of a cell which comprises contacting the cell with an antibody according to this invention under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of the mammalian SNORF36 receptor on the surface of the cell.

This invention still further provides a method of determining the physiological effects of varying levels of activity of mammalian SNORF36 receptors which comprises producing a

transgenic, nonhuman mammal in accordance with this invention whose levels of mammalian SNORF36 receptor activity are varied by use of an inducible promoter which regulates mammalian SNORF36 receptor expression.

This invention additionally provides a method of determining the physiological effects of varying levels of activity of mammalian SNORF36 receptors which comprises producing a panel of transgenic, nonhuman mammals in accordance with this invention each expressing a different amount of mammalian SNORF36 receptor.

Moreover, this invention provides method for identifying an antagonist capable of alleviating an abnormality wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor comprising administering a compound to a transgenic, nonhuman mammal according to this invention, and determining whether the compound alleviates any physiological and/or behavioral abnormality displayed by the transgenic, nonhuman mammal as a result of overactivity of a mammalian SNORF36 receptor, the alleviation of such an abnormality identifying the compound as an antagonist. In an embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In a further embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor. The invention also provides an antagonist identified by the preceding method according to this invention. This invention further provides a composition, e.g. a pharmaceutical composition comprising an antagonist according to this invention and a carrier, e.g. a pharmaceutically acceptable carrier. This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition according to this invention so as to thereby

treat the abnormality.

In addition, this invention provides a method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor comprising administering a compound to a transgenic, nonhuman mammal according to this invention, and determining whether the compound alleviates any physiological and/or behavioral abnormality displayed by the transgenic, nonhuman mammal, the alleviation of such an abnormality identifying the compound as an agonist. In an embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In a further embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor. This invention provides an agonist identified by the preceding method according to this invention. This invention provides a composition, e.g. a pharmaceutical composition comprising an agonist identified by a method according to this invention and a carrier, e.g. a pharmaceutically acceptable carrier.

Moreover, this invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition of this invention so as to thereby treat the abnormality.

Yet further, this invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific mammalian allele which comprises: (a) obtaining DNA of subjects suffering from the disorder; (b) performing a restriction digest of the DNA with a panel of restriction enzymes; (c) electrophoretically separating the resulting DNA fragments on a sizing gel; (d) contacting the resulting gel with a nucleic acid probe capable of

specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a mammalian SNORF36 receptor and labeled with a detectable marker; (e) detecting labeled bands which have hybridized to the DNA encoding a mammalian SNORF36 receptor to create a unique band pattern specific to the DNA of subjects suffering from the disorder; (f) repeating steps (a)-(e) with DNA obtained for diagnosis from subjects not yet suffering from the disorder; and (g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step (e) with the band pattern from step (f) for subjects not yet suffering from the disorder so as to determine whether the patterns are the same or different and thereby diagnose predisposition to the disorder if the patterns are the same.

In one embodiment, the disorder is a disorder associated with the activity of a specific mammalian allele is diagnosed.

This invention also provides a method of preparing a purified mammalian SNORF36 receptor according to this invention which comprises: (a) culturing cells which express the mammalian SNORF36 receptor; (b) recovering the mammalian SNORF36 receptor from the cells; and (c) purifying the mammalian SNORF36 receptor so recovered.

This invention further provides a method of preparing a purified mammalian SNORF36 receptor according to this invention which comprises: (a) inserting a nucleic acid encoding the mammalian SNORF36 receptor into a suitable expression vector; (b) introducing the resulting vector into a suitable host cell; (c) placing the resulting host cell in suitable condition permitting the production of the mammalian SNORF36 receptor; (d) recovering the mammalian SNORF36 receptor so produced; and optionally (e) isolating and/or purifying the mammalian SNORF36 receptor so recovered.

Furthermore, this invention provides a process for determining whether a chemical compound is a mammalian SNORF36 receptor agonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF36 receptor with the compound under conditions permitting the activation of the mammalian SNORF36 receptor, and detecting any increase in mammalian SNORF36 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF36 receptor agonist.

This invention also provides a process for determining whether a chemical compound is a mammalian SNORF36 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF36 receptor with the compound in the presence of a known mammalian SNORF36 receptor agonist, under conditions permitting the activation of the mammalian SNORF36 receptor, and detecting any decrease in mammalian SNORF36 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF36 receptor antagonist.

In an embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In another embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor.

This invention still further provides a composition, for example a pharmaceutical composition, which comprises an amount of a mammalian SNORF36 receptor agonist determined by a process according to this invention effective to increase activity of a mammalian SNORF36 receptor and a carrier, for example, a pharmaceutically acceptable carrier. In one embodiment, the mammalian SNORF36 receptor agonist is not previously known.

Also, this invention provides a composition, for example a

pharmaceutical composition, which comprises an amount of a mammalian SNORF36 receptor antagonist determined by a process according to this invention effective to reduce activity of a mammalian SNORF36 receptor and a carrier, for example, a pharmaceutically acceptable carrier. In one embodiment, the mammalian SNORF36 receptor antagonist is not previously known.

This invention moreover provides a process for determining whether a chemical compound specifically binds to and activates a mammalian SNORF36 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the chemical compound under conditions suitable for activation of the mammalian SNORF36 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change, e.g. an increase, in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian SNORF36 receptor.

In one embodiment, the second messenger response comprises chloride channel activation and the change in second messenger is an increase in the level of chloride current. In another embodiment, the second messenger response comprises change in intracellular calcium levels and the change in second messenger is an increase in the measure of intracellular calcium. In another embodiment, the second messenger response comprises release of inositol phosphate and the change in second messenger is an increase in the level of inositol phosphate. In another embodiment, the second messenger response comprises release of arachidonic acid and the change in second messenger is an increase in the level of arachidonic acid. In yet another embodiment, the second messenger response comprises GTPγS ligand binding and

the change in second messenger is an increase in GTPγS ligand binding. In another embodiment, the second messenger response comprises activation of MAP kinase and the change in second messenger response is an increase in MAP kinase activation. In a further embodiment, the second messenger response comprises cAMP accumulation and the change in second messenger response is a reduction in cAMP accumulation.

This invention still further provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian SNORF36 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with both the chemical compound and a second chemical compound known to activate the mammalian SNORF36 receptor, and with only the second chemical compound, under conditions suitable for activation of the mammalian SNORF36 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change, e.g. increase, in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian SNORF36 receptor.

In an embodiment of the present invention, the second chemical compound is a retinoic acid derivative. Examples of retinoic acid derivatives include, but are not limited to, all-*trans* retinoic acid (ATRA), 9-*cis*-retinal, 13-*cis*-retinal, and all-*trans*-retinal.

In one embodiment, the second messenger response comprises chloride channel activation and the change in second messenger response is a smaller increase in the level of chloride current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the second messenger response comprises change in intracellular calcium levels and the change in second messenger response is a smaller increase in the measure of intracellular calcium in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the second messenger response comprises release of inositol phosphate and the change in second messenger response is a smaller increase in the level of inositol phosphate in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

In one embodiment, the second messenger response comprises activation of MAP kinase and the change in second messenger response is a smaller increase in the level of MAP kinase activation in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the second messenger response comprises change in cAMP levels and the change in second messenger response is a smaller change in the level of cAMP in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the second messenger response comprises release of arachidonic acid and the change in second messenger response is an increase in the level of arachidonic acid levels in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In a further embodiment, the

second messenger response comprises GTPγS ligand binding and the change in second messenger is a smaller increase in GTPγS ligand binding in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

In one embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In a further embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor. In another embodiment, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In another embodiment, the mammalian cell is nonneuronal in origin. In another embodiment, the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell. In another embodiment, the compound is not previously known to bind to a mammalian SNORF36 receptor.

Further, this invention provides a compound determined by a process according to this invention and a composition, for example, a pharmaceutical composition, which comprises an amount of a mammalian SNORF36 receptor agonist determined to be such by a process according to this invention effective to increase activity of the mammalian SNORF36 receptor and a carrier, for example, a pharmaceutically acceptable carrier. In one embodiment, the mammalian SNORF36 receptor agonist is not previously known.

This invention also provides a composition, for example, a pharmaceutical composition, which comprises an amount of a mammalian SNORF36 receptor antagonist determined to be such by a process according to this invention, effective to reduce activity of the mammalian SNORF36 receptor and a carrier, for example a pharmaceutically acceptable carrier. In one embodiment, the mammalian SNORF36 receptor antagonist is not

previously known.

This invention yet further provides a method of screening a plurality of chemical compounds not known to activate a mammalian SNORF36 receptor to identify a compound which activates the mammalian SNORF36 receptor which comprises: (a) contacting cells transfected with and expressing the mammalian SNORF36 receptor with the plurality of compounds not known to activate the mammalian SNORF36 receptor, under conditions permitting activation of the mammalian SNORF36 receptor; (b) determining whether the activity of the mammalian SNORF36 receptor is increased in the presence of one or more of the compounds; and if so (c) separately determining whether the activation of the mammalian SNORF36 receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian SNORF36 receptor. In one embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In a further embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor.

This invention provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian SNORF36 receptor to identify a compound which inhibits the activation of the mammalian SNORF36 receptor, which comprises: (a) contacting cells transfected with and expressing the mammalian SNORF36 receptor with the plurality of compounds in the presence of a known mammalian SNORF36 receptor agonist, under conditions permitting activation of the mammalian SNORF36 receptor; (b) determining whether the extent or amount of activation of the mammalian SNORF36 receptor is reduced in the presence of one or more of the compounds, relative to the extent or amount of activation of the mammalian SNORF36 receptor in the absence of such one or more compounds; and if so (c) separately determining whether

each such compound inhibits activation of the mammalian SNORF36 receptor for each compound included in the plurality of compounds, so as to thereby identify any compound included in such plurality of compounds which inhibits the activation of the mammalian SNORF36 receptor.

In one embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In a further embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor. In another embodiment, wherein the cell is a mammalian cell. In another embodiment, the mammalian cell is non-neuronal in origin. In another embodiment, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell or an NIH-3T3 cell.

This invention also provides a composition, for example, a pharmaceutical composition, comprising a compound identified by a method according to this invention in an amount effective to increase mammalian SNORF36 receptor activity and a carrier, for example, a pharmaceutically acceptable carrier.

This invention still further provides a composition, for example, a pharmaceutical composition, comprising a compound identified by a method according to this invention in an amount effective to decrease mammalian SNORF36 receptor activity and a carrier, for example, a pharmaceutically acceptable carrier.

Furthermore, this invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject a compound which is a mammalian SNORF36 receptor agonist in an amount effective to treat the abnormality. In one embodiment, the abnormality is a regulation of a steroid

hormone disorder, an epinephrine release disorder, a gastrointestinal disorder, a cardiovascular disorder, an electrolyte balance disorder, hypertension, diabetes, a respiratory disorder, asthma, a reproductive function disorder, an immune disorder, an endocrine disorder, a musculoskeletal disorder, a neuroendocrine disorder, a cognitive disorder, a memory disorder, somatosensory and neurotransmission disorders, metabolic disorders, a motor coordination disorder, a sensory integration disorder, a motor integration disorder, a dopaminergic function disorder, an appetite disorder, such as anorexia or obesity, a sensory transmission disorder, drug addiction, an olfaction disorder, an autonomic nervous system disorder, pain, neuropsychiatric disorders, affective disorder, migraine, circadian disorders, visual disorders, urinary disorders, blood coagulation-related disorders, developmental disorders, or ischemia-reperfusion injury-related diseases.

In a further embodiment of the present invention, the abnormality is a gestational abnormality, a sleep disorder such as insomnia, jet lag or shift-related conditions, disorders associated with melatonin release, and disorders associated with choroid plexus function.

This invention additionally provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject a compound which is a mammalian SNORF36 receptor antagonist in an amount effective to treat the abnormality. In one embodiment, the abnormality is a regulation of a steroid hormone disorder, an epinephrine release disorder, a gastrointestinal disorder, a cardiovascular disorder, an electrolyte balance disorder, hypertension, diabetes, a respiratory disorder, asthma, a reproductive function disorder, an immune disorder, an endocrine disorder, a

musculoskeletal disorder, a neuroendocrine disorder, a cognitive disorder, a memory disorder, somatosensory and neurotransmission disorders, metabolic disorders, a motor coordination disorder, a sensory integration disorder, a motor integration disorder, a dopaminergic function disorder, an appetite disorder, such as anorexia or obesity, a sensory transmission disorder, drug addiction, an olfaction disorder, an autonomic nervous system disorder, pain, neuropsychiatric disorders, affective disorder, migraine, circadian disorders, visual disorders, urinary disorders, blood coagulation-related disorders, developmental disorders, or ischemia-reperfusion injury-related diseases.

In a further embodiment of the present invention, the abnormality is a gestational abnormality, a sleep disorder such as insomnia, jet lag or shift-related conditions, disorders associated with melatonin release, and disorders associated with choroid plexus function.

This invention also provides a process for making a composition of matter which specifically binds to a mammalian SNORF36 receptor which comprises identifying a chemical compound using a process in accordance with this invention and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof. In one embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In another embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor.

This invention further provides a process for preparing a composition, for example a pharmaceutical composition which comprises admixing a carrier, for example, a pharmaceutically acceptable carrier, and a pharmaceutically effective amount of a chemical compound identified by a process in accordance with this invention or a novel structural and functional

analog or homolog thereof. In one embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In another embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor.

Thus, once the gene for a targeted receptor subtype is cloned, it is placed into a recipient cell which then expresses the targeted receptor subtype on its surface. This cell, which expresses a single population of the targeted human receptor subtype, is then propagated resulting in the establishment of a cell line. This cell line, which constitutes a drug discovery system, is used in two different types of assays: binding assays and functional assays. In binding assays, the affinity of a compound for both the receptor subtype that is the target of a particular drug discovery program and other receptor subtypes that could be associated with side effects are measured. These measurements enable one to predict the potency of a compound, as well as the degree of selectivity that the compound has for the targeted receptor subtype over other receptor subtypes. The data obtained from binding assays also enable chemists to design compounds toward or away from one or more of the relevant subtypes, as appropriate, for optimal therapeutic efficacy. In functional assays, the nature of the response of the receptor subtype to the compound is determined. Data from the functional assays show whether the compound is acting to inhibit or enhance the activity of the receptor subtype, thus enabling pharmacologists to evaluate compounds rapidly at their ultimate human receptor subtypes targets permitting chemists to rationally design drugs that will be more effective and have fewer or substantially less severe side effects than existing drugs.

Approaches to designing and synthesizing receptor subtype-selective compounds are well known and include traditional medicinal chemistry and the newer technology of combinatorial

chemistry, both of which are supported by computer-assisted molecular modeling. With such approaches, chemists and pharmacologists use their knowledge of the structures of the targeted receptor subtype and compounds determined to bind and/or activate or inhibit activation of the receptor subtype to design and synthesize structures that will have activity at these receptor subtypes.

Combinatorial chemistry involves automated synthesis of a variety of novel compounds by assembling them using different combinations of chemical building blocks. The use of combinatorial chemistry greatly accelerates the process of generating compounds. The resulting arrays of compounds are called libraries and are used to screen for compounds ("lead compounds") that demonstrate a sufficient level of activity at receptors of interest. Using combinatorial chemistry it is possible to synthesize "focused" libraries of compounds anticipated to be highly biased toward the receptor target of interest.

Once lead compounds are identified, whether through the use of combinatorial chemistry or traditional medicinal chemistry or otherwise, a variety of homologs and analogs are prepared to facilitate an understanding of the relationship between chemical structure and biological or functional activity. These studies define structure activity relationships which are then used to design drugs with improved potency, selectivity and pharmacokinetic properties. Combinatorial chemistry is also used to rapidly generate a variety of structures for lead optimization. Traditional medicinal chemistry, which involves the synthesis of compounds one at a time, is also used for further refinement and to generate compounds not accessible by automated techniques. Once such drugs are defined the production is scaled up using standard chemical manufacturing methodologies utilized throughout the pharmaceutical and chemistry industry.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

EXPERIMENTAL DETAILS

Materials and Methods

Identification of a fragment of the human SNORF36 receptor

A human placental genomic phage library (2.75 million recombinants, Stratagene, LaJolla, CA) was screened using ^{32}P-labeled oligonucleotide probes, RW76, RW77, RW96, RW97, RW98 and RW99, designed against transmembrane (TM) domains III, V and VI of the human serotonin 5-$HT1_D$ receptor. The overlapping oligonucleotide probes were labeled with DNA Polymerase I (Klenow Fragment) and [α-^{32}P]dATP/dCTP.

Hybridization of nitrocellulose filter overlays of the plates was performed at low stringency: 40°C in a solution containing 25% formamide, 5x SSC (1X SSC is 0.15M sodium chloride, 0.015M sodium citrate), 1x Denhardt's solution (0.02% polyvinylpyrrolindone, 0.02% Ficoll, 0.02% bovine serum albumin), 7 mM Tris and 25 µg/ml sonicated salmon sperm DNA. The filters were washed at 40 °C in 0.1x SSC containing 0.1% sodium dodecyl sulfate and exposed at -70°C to Kodak XAR film in the presence of intensifying screens.

A positive signal on plate 10 was isolated on a secondary plating. A 2.3 kb fragment, from a *Hind*III/*Xba*I digest of DNA isolated from this positive, was identified by Southern blot analysis, subcloned into pUC18 (Gibco BRL, Gaithersburg, MD) and used to transform E.Coli XL1 Blue cells (Stratagene, La Jolla, CA). Plasmid DNA from one transformant, K39, was sequenced using the Sanger dideoxy nucleotide chain termination method (Sanger et al., 1977) on denatured double-stranded plasmid templates, using Sequenase (US Biochemical Corp., Cleveland, OH). Analysis of the sequence of K39 revealed TMs III and IV of a novel GPCR with highest homology to adrenergic receptors.

Isolation of a full-length human SNORF36 receptor

To isolate a full-length SNORF36 receptor, pools of a human hippocampal cDNA library were screened by polymerase chain reaction (PCR) using T604, a K39 specific primer, and either T94, a vector primer, or T603, a K39 specific primer. PCR was performed with Taq DNA Polymerase (Roche Molecular Biochemicals, Indianapolis, IN) with the following protocol: 94°C hold for 5 minutes; 40 cycles of 94°C for 2 minute, 72°C for 4 minutes; 10 minute hold at 72°C; 4°C hold until the samples are run on a gel. High stringency hybridization of isolated colonies from two positive pools with T605, a K39-specific oligonucleotide probe, and subsequent PCR testing of positive colonies, resulted in the isolation of a single positive clone named TL252. Analysis of the sequence of TL252 revealed that it contained TMI-TMVII, but was missing the NH_2 and COOH termini.

To isolate the COOH termini of SNORF36, 3' Rapid Amplification of cDNA Ends (RACE), was performed using the Clontech Marathon cDNA Amplification kit (Clontech, Palo Alto, CA). Using the supplier's protocol, Marathon adapters were ligated onto ds cDNA prepared from human hippocampal polyA+ RNA. The initial PCR was performed with the supplier's Adapter Primer 1 and A48, a forward primer from TMVI of TL252. 2 µls of this initial PCR reaction was re-amplified using the Adaptor Primer 2 and A49, a forward primer from TMVI. PCR was performed with Advantage Klentaq Polymerase (Clontech, Palo Alto, CA) under the following conditions: 30 seconds at 94° C; 30 cycles of 94° C for 30 seconds, 68° C 4 minutes; and 4° C hold until the products were ready for analysis. A 1.5 kb fragment was isolated from an agarose TAE gel using the QIAQUICK gel extraction kit (QIAGEN, Chatsworth, CA) and subcloned into the TA cloning vector (Invitrogen, San Diego, CA). One transformant, AB25, was sequenced using the ABI Big Dye cycle sequencing protocol and

ABI 377 sequencers (ABI, Foster City, CA). Sequences were analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, WI.). Analysis of the sequence of AB25 revealed the COOH terminus and a stop codon approximately 350 bp downstream from TMVII.

To look for a full-length SNORF36 cDNA, pools of a human hippocampal cDNA library were screened by PCR with TL252-specific primers BB788 and BB789 using the Expand Long Template PCR System (Boehringer-Mannheim, Indianapolis, IN). Conditions for PCR amplification were as follows: 94°C hold for 5 minutes; 40 cycles of 94°C for 30 seconds, 68°C for 2 minutes; 68°C hold for 5 minutes; 4°C hold until ready for agarose gel electrophoresis. This screen yielded three positive pools. Subsequent high-stringency hybridization of isolated colonies from two of these pools using a [γ-^{32}P]-ATP-labeled SNORF36-specific probe (BB791) resulted in the identification of 2 positive individual colonies, 260.13.1 and 243.33.3. Sequencing of these clones revealed that they were identical and were full-length at the 3' end. However, while these clones contained some sequence upstream of TMI, they did not contain the initiating methionine. In addition, these clones both contained an 11 amino acid insert in the first intracellular loop that was not present in TL252.

To identify the NH_2 terminal sequence, 5'RACE was performed on Marathon-Ready human hippocampal cDNA (Clontech, Palo Alto, CA) according to the Marathon cDNA Amplification Kit protocol. The initial PCR was performed with the supplier's Adapter Primer 1 and BB798 a reverse primer from the second intracellular loop of 260.13.1. One µl of this initial PCR reaction was re-amplified using the Adaptor Primer 2 and BB797, a reverse primer from TMI. PCR was performed with Advantage Klentaq Polymerase (Clontech, Palo Alto, CA) under the following conditions: 30 seconds at 94°C; 5 cycles of 94°C for 30 seconds and 72°C for 3 minutes; 5 cycles of 94°C

for 30 seconds and 70°C for 3 minutes; 23 cycles (initial PCR) or 20 cycles (nested PCR) of 94°C for 30 seconds and 68°C for 3 minutes; 68°C hold for 7 minutes, and 4°C hold until the products were ready for analysis. A 500 bp fragment was isolated from a 1% agarose TAE gel using the QIAQUICK gel extraction kit (QIAGEN, Chatsworth, CA) and sequenced using ABI 377 sequencers and BigDye termination cycle sequencing as described above. Analysis of this sequence revealed the NH_2 terminus including 2 methionines 71 and 52 amino acids upstream from TMI.

A full-length cDNA clone for SNORF36a (without the 11 amino acid insert) was constructed as follows. The 5' end of the cDNA was amplified from hippocampal and pituitary cDNAs using the Expand Long Template PCR System (Roche Molecular Biochemicals, Indianapolis, IN) and BB812, a forward primer from the 5'UT also incorporating a *BamH*I restriction site, and BB813, a reverse primer from the third extracellular loop. Conditions for PCR amplification were as follows: 94°C hold for 5 minutes; 37 cycles of 94°C for 30 seconds; 68°C for 2.5 minutes; 68°C hold for 7 minutes; 4°C hold until ready for agarose gel electrophoresis. 1050 bp bands from 9 independent PCR reactions were cut from a 1% agarose gel, purified using the QIAQUICK gel extraction kit (QIAGEN, Chatsworth, CA), subcloned into the TA cloning vector (Invitrogen, San Diego, CA) and sequenced using the ABI Big Dye cycle sequencing protocol and ABI 377 sequencers (ABI, Foster City, CA). Analysis of these sequences revealed the presence of an allelic variation. Nucleotide 39 (Figures 1A-C and 3A-C) is either an adenine or a guanine. One of these PCR products, F9, matched the consensus sequence with the exception of a single conservative nucleotide change. A 1021 bp *BamH*I/*Bgl*I fragment from F9 was then ligated along with a 1180 bp *Bgl*I/*EcoR*I fragment from the human hippocampal library pool 260.13.1 into a *BamH*I/*EcoR*I-cut pcDNA3.1

(Invitrogen, San Diego, CA), and this construct was named BO108. The single point mutation introduced by the PCR fragment was corrected with the QuikChange Site-Directed Mutagenesis Kit (Stratagene, LaJolla, CA) using BB926 and BB927 and the manufacturer's instructions. The resulting SNORF36a construct, BO109, was sequenced on both strands as described above. This clone was renamed pcDNA3.1-hSNORF36a-f. A full-length clone for SNORF36b (with the 11 amino acid insert) was constructed as follows. A 1260 bp fragment was obtained by amplifying BO108 with BB796, a forward primer in TMI and BB936, a reverse primer at the stop codon also incorporating a *Hind*III site, with the following protocol: 94°C hold for 5 minutes; 32 cycles of 94°C for 30 seconds; 68°C for 2.5 minutes; 68°C hold for 7 minutes; 4°C hold until ready for agarose gel electrophoresis. A 1130 bp *Nsp*I/*Hind*III fragment from this PCR product and a 390 bp *BamHI*/*Nsp*I fragment from K81, one of the PCR products from the BB812-BB813 PCR described above, was ligated into a *BamHI*/*Hind*III-cut pcDNA3.1 (Invitrogen, San Diego, CA). The resulting SNORF36b construct, BO110, was sequenced on both strands as described above. This clone was renamed pcDNA3.1-hSNORF36b-f.

Isolation of a Fragment of the Rat Homologue of SNORF36

To obtain a fragment of the rat homologue of SNORF36, 100 ng of rat genomic DNA (Clontech, Palo Alto, CA) was amplified with BB788, a forward PCR primer corresponding to TMIII of human SNORF36 and BB1097, a reverse primer corresponding to TMV of human SNORF36. PCR was performed with the Expand Long Template PCR System (Roche Molecular Biochemicals) under the following conditions: 30 seconds at 94°C, 45 seconds at 45°C or 50°C, 1.5 minutes at 68°C for 40 cycles, with a pre- and post-incubation of 5 minutes at 94°C and 7 minutes at 68°C respectively. Bands of 800 bp from 7 independent PCR reactions were isolated from a TAE gel, purified using the

QIAQUICK gel extraction kit (QIAGEN, Chatsworth, CA), and sequenced on both strands as described above. Analysis of the sequence revealed an intron in the second extracellular loop. The consensus of the 7 sequences was used to design forward and reverse PCR primers (BB1182, also incorporating a *BamH*I restriction site, and BB1183, also incorporating a *Hind*III site) which were used to amplify a band from rat spinal cord cDNA using the following conditions: 30 seconds at 94°C, 30 seconds at 64°C, 1.5 minutes at 68°C for 37 cycles, with a pre- and post-incubation of 5 minutes at 94°C and 7 minutes at 68°C respectively. Products from 3 independent PCR reactions were digested with *BamH*I and *Hind*III and fragments of 250 bp were gel-purified and ligated into the expression vector pEXJ.T3T7. One transformant from each PCR reaction was sequenced as above. The nucleotide sequences of two products were identical to the consensus and one, KO56, was renamed pEXJ-rSNORF36-p.

Isolation of a full-length rat SNORF36 receptor

To look for the full-length rat SNORF36 cDNA, pools of a rat spinal cord cDNA library were screened by PCR with BB1182, a forward primer from TMIII of the rat SNORF36 fragment, and BB1183, a reverse primer from TMV of the rat SNORF36 fragment. PCR was performed using the Expand Long Template PCR System (Roche Molecular Biochemicals, Indianapolis, IN) under the following conditions: 94°C hold for 5 minutes; 40 cycles of 94°C for 30 seconds, 68°C for 5 minutes; 68°C hold for 7 minutes; 4°C hold until ready for agarose gel electrophoresis. This screen yielded 2 positive pools. Subsequent high-stringency hybridization of isolated colonies from 1 of these pools using a [γ-^{32}P]-ATP-labeled rat SNORF36-specific probe (BB1298) resulted in the identification of a positive individual colony, N323.16.1F, renamed KO109. Sequencing of KO109 revealed that it contained an insert of 2.6 kb, including an open reading

frame of 1422 nucleotides, 148 nucleotides of 5'UT and approximately 1000 nucleotides of 3'UT. This clone was renamed pEXJ.T7-rSNORF36-f.

Isolation of other species homologs of SNORF36 receptor cDNA

A nucleic acid sequence encoding a SNORF36 receptor cDNA from other species may be isolated using standard molecular biology techniques and approaches such as those described below:

Approach #1: A genomic library (e.g., cosmid, phage, P1, BAC, YAC) generated from the species of interest may be screened with a ^{32}P-labeled oligonucleotide probe corresponding to a fragment of the human or rat SNORF36 receptors whose sequence is shown in Figures 1A-1C, 3A-3C, 5 or 9A-9C to isolate a genomic clone. The full-length sequence may be obtained by sequencing this genomic clone. If one or more introns are present in the gene, the full-length intronless gene may be obtained from cDNA using standard molecular biology techniques. For example, a forward PCR primer designed in the 5'UT and a reverse PCR primer designed in the 3'UT may be used to amplify a full-length, intronless receptor from cDNA. Standard molecular biology techniques could be used to subclone this gene into a mammalian expression vector.

Approach #2: Standard molecular biology techniques may be used to screen commercial cDNA phage libraries of the species of interest by hybridization under reduced stringency with a ^{32}P-labeled oligonucleotide probe corresponding to a fragment of the sequences shown in Figures 1A-1C, 3A-3C, 5 or 9A-9C. One may isolate a full-length SNORF36 receptor by obtaining a plaque purified clone from the lambda libraries and then subjecting the clone to direct DNA sequencing. Alternatively, standard molecular biology techniques could be used to screen cDNA plasmid libraries by PCR amplification

of library pools using primers designed against a partial species homolog sequence. A full-length clone may be isolated by Southern hybridization of colony lifts of positive pools with a ^{32}P-oligonucleotide probe.

Approach #3: 3' and 5' RACE may be utilized to generate PCR products from cDNA derived from the species of interest expressing SNORF36 which contain the additional sequence of SNORF36. These RACE PCR products may then be sequenced to determine the additional sequence. This new sequence is then used to design a forward PCR primer in the 5'UT and a reverse primer in the 3'UT. These primers are then used to amplify a full-length SNORF36 clone from cDNA.

Examples of other species include, but are not limited to, mouse, dog, monkey, hamster and guinea pig.

Primers and probes used in the identification of SNORF36:

RW76: 5′- CATCGCCCTCGACGTGCTGTGCTGCACCTCATCCATCT TGCACCT -3′ (SEQ ID NO: 9)

RW77: 5′- CATGGACAGGTCGCGCTACCGCGTGTCCACGTTCTACC TACTCCA -3′ (SEQ ID NO: 10)

RW96: 5′- GGCATCATCATGGGCACCTTCATCCTCTGCTGGCTGCC CTTCTTC -3′ (SEQ ID NO: 11)

RW97: 5′- GCAGAAGGGCAGAACAAGAGCCACGATGAAGAAGGGCA GCCAGCA -3′ (SEQ ID NO: 12)

RW98: 5′- TGGCTGTCATCGGACATCACTTGTTGCACTGCCTCCAT CCTGCAC -3′ (SEQ ID NO: 13)

RW99: 5′- GTAGCGGTCCAGGGCGATGACACAGAGGTGCAGGATGG AGGCAGT -3′ (SEQ ID NO: 14)

T604: 5′- CCAGCCGAAGAAGGGTGGCAGACTCCA -3′ (SEQ ID NO: 15)

T94: 5′- CTTCTAGGCCTGTACGGAAGTGTTA -3′ (SEQ ID NO: 16)

T603: 5′- GCACAGGCTGCGAGTTCTATTCCTT -3′ (SEQ ID NO: 17)

T605: 5′- CTGGTAATCACACACCCGCTGGCCACCTTTGGTGTGGC GTCCAAG -3′ (SEQ ID NO: 18)

A48: 5'- AGATCATGCTGCTGGTCATCCTCC -3' (SEQ ID NO: 19)

A49: 5'- TCGTGCTCTCCTGGGCTCCCT -3' (SEQ ID NO: 20)

BB788: 5'- TCCTCCATGATCACCCTGACGGC -3' (SEQ ID NO: 21)

BB789: 5'- TCTGGAGAGCCCGTCCTGTCTCC -3' (SEQ ID NO: 22)

BB791: 5'- CGGCCGTGCGTGCCTACACCATGCTTCTCTGCTGCTTC GTGTTCTTCC -3' (SEQ ID NO: 23)

BB798: 5'- TTGGACGCCACACCAAAGGTGGCC -3' (SEQ ID NO: 24)

BB797: 5'- GGTATAGATGACCGTCAGGTTGCC -3' (SEQ ID NO: 25)

BB812: 5'-CGAACAGGATCCTCTCTGTGGGCTCGAGCAAGGACC -3' (SEQ ID NO: 26)

BB813: 5'- ACGTGTGCGTACCCAGCAAAGGCC -3' (SEQ ID NO: 27)

BB926: 5'- GTCCCACAGCACCTGGGACTTGGGCTGC -3' (SEQ ID NO: 28)

BB927: 5'- GCAGCCCAAGTCCCAGGTGCTGTGGGAC -3' (SEQ ID NO: 29)

BB796: 5'- GGCAACCTGACGGTCATCTATACC -3' (SEQ ID NO: 30)

BB936: 5'- CAGCATAAGCTTCCAGTGGGCGTCCTACATCCTGG -3' (SEQ ID NO: 31)

BB1097: 5'- CAGTAGATGATGATAAGCAGAGG -3' (SEQ ID NO: 32)

BB1182: 5'- CGAACAGGATCCCATAGCCATGGACCGCTATCTGG -3' (SEQ ID NO: 33)

BB1183: 5'- CCTAGCAAGCTTGAGGAAGAAGACAAAGCAGAAGAGC -3' (SEQ ID NO: 34)

BB1298: 5'-CGGACGGCACTAGTCCTGCTAGGTGTCTGGCTCTATGCCCTGG CCTGG -3' (SEQ ID NO: 35)

Host cells

A broad variety of host cells can be used to study heterologously expressed proteins. These cells include, but are not limited to, mammalian cell lines such as: COS-7, CHO, LM(*tk*-), HEK293, etc.; insect cell lines such as Sf9, Sf21, Trichoplusia ni 5B-4, etc.; amphibian cells such as *Xenopus* oocytes; assorted yeast strains; assorted bacterial cell strains; and others. Culture conditions for each of these cell types are specific and are known to those familiar with

the art. The cells used to express human SNORF36 receptor were COS-7 and Human embryonic kidney (HEK) 293 cells.

COS-7 cells are grown on 150 mm plates in DMEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 µg/ml streptomycin) at 37°C, 5% CO_2. Stock plates of COS-7 cells are trypsinized and split 1:6 every 3-4 days.

HEK293 cells are grown on 150 mm plates in DMEM with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 µg/ml streptomycin) at 37°C, 5% CO_2. Stock plates of 293 cells are trypsinized and split 1:6 every 3-4 days.

Transient expression

DNA encoding proteins to be studied can be transiently expressed in a variety of mammalian, insect, amphibian and other cell lines by several methods, such as, calcium phosphate-mediated, DEAE-dextran mediated, liposomal-mediated, viral-mediated, electroporation-mediated and microinjection delivery. Each of these methods may require optimization of assorted experimental parameters depending on the DNA, cell line, and the type of assay to be subsequently employed.

A typical protocol for the DEAE-dextran method as applied to Cos-7 and HEK293 cells is described as follows. Cells to be used for transfection are split 24 hours prior to the transfection to provide flasks which are 70-80% confluent at the time of transfection. Briefly, 8 µg of receptor DNA plus 8 µg of any additional DNA needed (e.g. G_α protein expression vector, reporter construct, antibiotic resistance marker, mock vector, etc.) are added to 9 ml of complete DMEM plus DEAE-dextran mixture (10 mg/ml in PBS). Cells plated into

a T225 flask (sub-confluent) are washed once with PBS and the DNA mixture is added to each flask. The cells are allowed to incubate for 30 minutes at 37°C, 5% CO_2. Following the incubation, 36 ml of complete DMEM with 80 µM chloroquine is added to each flask and allowed to incubate an additional 3 hours. The medium is then aspirated and 24 ml of complete medium containing 10% DMSO for exactly 2 minutes and then aspirated. The cells are then washed 2 times with PBS and 30 ml of complete DMEM added to each flask. The cells are then allowed to incubate over night. The next day the cells are harvested by trypsinization and reseeded into 96 well plates.

<u>Stable expression</u>

Heterologous DNA can be stably incorporated into host cells, causing the cell to perpetually express a foreign protein. Methods for the delivery of the DNA into the cell are similar to those described above for transient expression but require the co-transfection of an ancillary gene to confer drug resistance on the targeted host cell. The ensuing drug resistance can be exploited to select and maintain cells that have taken up the DNA. An assortment of resistance genes are available including but not restricted to neomycin, kanamycin, and hygromycin. For purposes of studies concerning the receptor of this invention, stable expression of a heterologous receptor protein is typically carrier out in, mammalian cells including but not necessarily restricted to, CHO, HEK293, LM(tk-), etc. In addition native cell lines that naturally carry and express the nucleic acid sequences for the receptor may be used without the need to engineer the receptor complement.

<u>Functional assays</u>

Cells expressing the receptor DNA of this invention may be used to screen for ligands to said receptor using functional

assays. Once a ligand is identified the same assays may be used to identify agonists or antagonists of the receptor that may be employed for a variety of therapeutic purposes. It is well known to those in the art that the over-expression of a GPCR can result in the constitutive activation of intracellular signaling pathways. In the same manner, over-expression of the SNORF36a receptor in any cell line as described above, can result in the activation of the functional responses described below, and any of the assays herein described can be used to screen for agonist, partial agonist, inverse agonist and antagonist ligands of the SNORF36 receptor.

A wide spectrum of assays can be employed to screen for the presence of SNORF36 receptor ligands. These assays range from traditional measurements of total inositol phosphate accumulation, cAMP levels, intracellular calcium mobilization, and potassium currents, for example; to systems measuring these same second messengers but which have been modified or adapted to be of higher throughput, more generic and more sensitive; to cell based assays reporting more general cellular events resulting from receptor activation such as metabolic changes, differentiation, cell division/proliferation. Description of several such assays follow.

Cyclic AMP (cAMP) assay

The receptor-mediated stimulation or inhibition of cyclic AMP (cAMP) formation may be assayed in cells expressing the receptors. Cells are plated in 96-well plates or other vessels and preincubated in a buffer such as HEPES buffered saline (NaCl (150 mM), $CaCl_2$ (1 mM), KCl (5 mM), glucose (10 mM)) supplemented with a phosphodiesterase inhibitor such as 5mM theophylline, with or without protease inhibitor cocktail (For example, a typical inhibitor cocktail contains 2 µg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 µg/ml phosphoramidon.)

for 20 min at 37°C, in 5% CO_2. Test compounds are added with or without 10mM forskolin and incubated for an additional 10 min at 37°C. The medium is then aspirated and the reaction stopped by the addition of 100 mM HCl or other methods. The plates are stored at 4°C for 15 min, and the cAMP content in the stopping solution is measured by radioimmunoassay. Radioactivity may be quantified using a gamma counter equipped with data reduction software. Specific modifications may be performed to optimize the assay for the receptor or to alter the detection method of cAMP.

Arachidonic acid release assay

Cells expressing the receptor are seeded into 96 well plates or other vessels and grown for 3 days in medium with supplements. ^{3}H-arachidonic acid (specific activity = 0.75 µCi/ml) is delivered as a 100 µL aliquot to each well and samples are incubated at 37° C, 5% CO_2 for 18 hours. The labeled cells are washed three times with medium. The wells are then filled with medium and the assay is initiated with the addition of test compounds or buffer in a total volume of 250 µL. Cells are incubated for 30 min at 37°C, 5% CO_2. Supernatants are transferred to a microtiter plate and evaporated to dryness at 75°C in a vacuum oven. Samples are then dissolved and resuspended in 25 µL distilled water. Scintillant (300 µL) is added to each well and samples are counted for ^{3}H in a Trilux plate reader. Data are analyzed using nonlinear regression and statistical techniques available in the GraphPAD Prism package (San Diego, CA).

Intracellular calcium mobilization assays

The intracellular free calcium concentration may be measured by microspectrofluorimetry using the fluorescent indicator dye Fura-2/AM (Bush et al, 1991). Cells expressing the receptor are seeded onto a 35 mm culture dish containing a glass coverslip insert and allowed to adhere overnight. Cells are then washed with HBS and loaded with 100 µL of

Fura-2/AM (10 µM) for 20 to 40 min. After washing with HBS to remove the Fura-2/AM solution, cells are equilibrated in HBS for 10 to 20 min. Cells are then visualized under the 40X objective of a Leitz Fluovert FS microscope and fluorescence emission is determined at 510 nM with excitation wavelengths alternating between 340 nM and 380 nM. Raw fluorescence data are converted to calcium concentrations using standard calcium concentration curves and software analysis techniques.

In another method, the measurement of intracellular calcium can also be performed on a 96-well (or higher) format and with alternative calcium-sensitive indicators, preferred examples of these are: aequorin, Fluo-3, Fluo-4, Fluo-5, Calcium Green-1, Oregon Green, and 488 BAPTA. After activation of the receptors with agonist ligands the emission elicited by the change of intracellular calcium concentration can be measured by a luminometer, or a fluorescence imager; a preferred example of this is the fluorescence imager plate reader (FLIPR).

Cells expressing the receptor of interest are plated into clear, flat-bottom, black-wall 96-well plates (Costar) at a density of 30,000-80,000 cells per well and allowed to incubate over night at 5% CO_2, 37°C. The growth medium is aspirated and 100 µl of dye loading medium is added to each well. The loading medium contains: Hank's BSS (without phenol red)(Gibco), 20 mM HEPES (Sigma), 0.1% BSA (Sigma), dye/pluronic acid mixture (e.g. 1 mM Flou-3, AM (Molecular Probes), 10% pluronic acid (Molecular Probes); (mixed immediately before use), and 2.5 mM probenecid (Sigma)(prepared fresh)). The cells are allowed to incubate for about 1 hour at 5% CO_2, 37°C.

During the dye loading incubation the compound plate is prepared. The compounds are diluted in wash buffer (Hank's

BSS without phenol red), 20 mM HEPES, 2.5 mM probenecid to a 3X final concentration and aliquoted into a clear v-bottom plate (Nunc). Following the incubation the cells are washed to remove the excess dye. A Denley plate washer is used to gently wash the cells 4 times and leave a 100 µl final volume of wash buffer in each well. The cell plate is placed in the center tray and the compound plate is placed in the right tray of the FLIPR. The FLIPR software is setup for the experiment, the experiment is run and the data are collected. The data are then analyzed using an excel spreadsheet program.

Antagonist ligands are identified by the inhibition of the signal elicited by agonist ligands.

The intracellular free calcium (Ca^{2+}) concentration may be measured by the Fluorescence Imager Plate Reader (FLIPR™).

Cells transfected with appropriate DNA as described earlier were plated into clear, flat-bottom, black-wall 96-well plates (Costar) at a density of 80,000-150,000 cells per well and allowed to incubate for 24 hr at 5% CO_2, 37°C. Whenever necessary, the cells were placed in a waterbath maintained at 37°C and were exposed to a lamp light (50W) placed at a distance of approximately 30 cm, 90 to 120 min before the imaging. The growth medium was aspirated and 100 µl of loading medium containing fluo-3 dye was added to each well 60 min before the imaging. The loading medium contained: Hank's BSS (without phenol red)(Gibco), 20 mM HEPES (Sigma), 0.1 or 1% BSA (Sigma), dye/pluronic acid mixture (e.g. 1 mM Flou-3, AM (Molecular Probes) and 10% pluronic acid (Molecular Probes) mixed immediately before use), and 2.5 mM probenecid (Sigma)(prepared fresh). The cells were allowed to incubate for about 1 hour at 5% CO_2, 37°C.

Before or during the incubation of cells with the dye-loading

medium, the test compound plate was prepared. Since retinoids are chemically unstable and can undergo rapid photoisomerization (Hu et al., 1994), care was taken to weigh, dissolve and prepare appropriate concentrations of the test compounds under photographic darkroom lights. The test compounds were diluted in wash buffer (Hank's BSS (without phenol red), 20 mM HEPES, 2.5 mM probenecid) to a 4X final concentration and aliquoted into a clear v-bottom plate (Nunc). The test compounds were protected from light till their addition to cells. Following the incubation with the dye-loading medium, the cells were washed to remove the excess dye. A Denley plate washer was used to gently wash the cells 4 times and leave a 100 µl final volume of wash buffer in each well. The cell plate was placed in the center tray and the test compound plate was placed in the right tray of the FLIPR. The FLIPR software was setup for the experiment, the experiment was run and the data were collected. For the agonist experiment, the sampling rate in the FLIPR was every 1 sec for the first minute and every 2 sec for the next two minutes. In this paradigm, the test compound was added after recording base line for the first 10 sec. For the antagonist experiment, the sampling rate in the FLIPR was every 1 sec for the first minute, every 6 sec for the next 5 minutes, every 1 sec for the next one minute followed by every 2 sec next two minutes. To evaluate the antagonistic activity, the test compound was added to the cells 10 sec after commencing the recording and the agonist was added at 310 sec of the recording. Baseline subtraction and negative control corrections were performed on the traces. The collected data were then analyzed using an Excel spreadsheet program.

Inositol phosphate assay

Human SNORF36a receptor-mediated activation of the inositol phosphate (IP) second messenger pathways was assessed by radiometric measurement of IP products.

For example, in a 96 well microplate format assay, cells are plated at a density of 70,000 cells per well and allowed to incubate for 24 hours. The cells are then labeled with 0.5 μCi [^{3}H]myo-inositol overnight at 37^{0}C, 5% CO_2. Immediately before the assay, the medium is removed and replaced with 90 μL of PBS containing 10 mM LiCl. The plates are then incubated for 15 min at 37^{0}C, 5% CO_2. Following the incubation, the cells are challenged with agonist (10 μl/well; 10x concentration) for 30 min at 37^{0}C, 5% CO_2. The challenge is terminated by the addition of 100 μL of 50% v/v trichloroacetic acid, followed by incubation at 4^{0}C for greater than 30 minutes. Total IPs are isolated from the lysate by ion exchange chromatography. Briefly, the lysed contents of the wells are transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates are prepared adding 100 μL of Dowex AG1-X8 suspension (50% v/v, water: resin) to each well. The filter plates are placed on a vacuum manifold to wash or elute the resin bed. Each well is first washed 2 times with 200 μl of 5 mM myo-inositol. Total [^{3}H]inositol phosphates are eluted with 75 μl of 1.2 M ammonium formate/0.1 M formic acid solution into 96-well plates. 200 μL of scintillation cocktail is added to each well, and the radioactivity is determined by liquid scintillation counting.

Cells were plated at a density of 70,000 cells per well and allowed to incubate for 24 hours. The cells were then labeled with 0.5 μCi [^{3}H]myo-inositol overnight at 37^{0}C, 5% CO_2. Immediately before the assay, the medium was removed and replaced with 180 μL of Phosphate-Buffered Saline (PBS) containing 10 mM LiCl. The plates were then incubated for 20 min at 37^{0}C, 5% CO_2. Following the incubation, the cells were challenged with agonist (20 μl/well; 10x concentration) for 30 min at 37^{0}C and were simultaneously either exposed or not to a lamp light (50W) placed at approximately 30 cm distance from the cells. The challenge was terminated by the addition

of 100 µL of 5% v/v trichloroacetic acid, followed by incubation at 4°C for greater than 30 minutes. Total IPs were isolated from the lysate by ion exchange chromatography. Briefly, the lysed contents of the wells were transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates were prepared adding 100 µL of Dowex AG1-X8 suspension (50% v/v, water: resin) to each well. The filter plates were placed on a vacuum manifold to wash or elute the resin bed. Each well was first washed 2 times with 200 µl of 5 mM myo-inositol. Total [^{3}H]inositol phosphates were eluted with 75 µl of 1.2M ammonium formate/0.1M formic acid solution into 96-well plates. 200 µL of scintillation cocktail was added to each well, and the radioactivity was determined by liquid scintillation counting.

<u>GTPγS functional assay</u>

Membranes from cells expressing the receptor are suspended in assay buffer (e.g., 50 mM Tris, 100 mM NaCl, 5 mM $MgCl_2$, 10 µM GDP, pH 7.4) with or without protease inhibitors (e.g., 0.1% bacitracin). Membranes are incubated on ice for 20 minutes, transferred to a 96-well Millipore microtiter GF/C filter plate and mixed with GTPγ^{35}S (e.g., 250,000 cpm/sample, specific activity ~1000 Ci/mmol) plus or minus unlabeled GTPγS (final concentration = 100 µM). Final membrane protein concentration ≈ 90 µg/ml. Samples are incubated in the presence or absence of test compounds for 30 min. at room temperature, then filtered on a Millipore vacuum manifold and washed three times with cold (4°C) assay buffer. Samples collected in the filter plate are treated with scintillant and counted for ^{35}S in a Trilux (Wallac) liquid scintillation counter. It is expected that optimal results are obtained when the receptor membrane preparation is derived from an appropriately engineered heterologous expression system, i.e., an expression system resulting in high levels of expression of the receptor and/or expressing

G-proteins having high turnover rates (for the exchange of GDP for GTP). GTPγS assays are well-known to those skilled in the art, and it is contemplated that variations on the method described above, such as are described by Tian et al. (1994) or Lazareno and Birdsall (1993), may be used.

Microphysiometric assay

Because cellular metabolism is intricately involved in a broad range of cellular events (including receptor activation of multiple messenger pathways), the use of microphysiometric measurements of cell metabolism can in principle provide a generic assay of cellular activity arising from the activation of any orphan receptor regardless of the specifics of the receptor's signaling pathway.

General guidelines for transient receptor expression, cell preparation and microphysiometric recording are described elsewhere (Salon, J.A. and Owicki, J.A., 1996). Typically cells expressing receptors are harvested and seeded at 3 x 10^5 cells per microphysiometer capsule in complete media 24 hours prior to an experiment. The media is replaced with serum free media 16 hours prior to recording to minimize non-specific metabolic stimulation by assorted and ill-defined serum factors. On the day of the experiment the cell capsules are transferred to the microphysiometer and allowed to equilibrate in recording media (low buffer RPMI 1640, no bicarbonate, no serum (Molecular Devices Corporation, Sunnyvale, CA) containing 0.1% fatty acid free BSA), during which a baseline measurement of basal metabolic activity is established.

A standard recording protocol specifies a 100 µl/min flow rate, with a 2 min total pump cycle which includes a 30 sec flow interruption during which the acidification rate measurement is taken. Ligand challenges involve a 1 min 20 sec exposure to the sample just prior to the first post

challenge rate measurement being taken, followed by two additional pump cycles for a total of 5 min 20 sec sample exposure. Typically, drugs in a primary screen are presented to the cells at 10 μM final concentration.

Follow up experiments to examine dose-dependency of active compounds are then done by sequentially challenging the cells with a drug concentration range that exceeds the amount needed to generate responses ranging from threshold to maximal levels. Ligand samples are then washed out and the acidification rates reported are expressed as a percentage increase of the peak response over the baseline rate observed just prior to challenge.

MAP kinase assay

MAP kinase (mitogen activated kinase) may be monitored to evaluate receptor activation. MAP kinase is activated by multiple pathways in the cell. A primary mode of activation involves the ras/raf/MEK/MAP kinase pathway. Growth factor (tyrosine kinase) receptors feed into this pathway via SHC/Grb-2/SOS/ras. Gi coupled receptors are also known to activate ras and subsequently produce an activation of MAP kinase. Receptors that activate phospholipase C (such as Gq/G11-coupled) produce diacylglycerol (DAG) as a consequence of phosphatidyl inositol hydrolysis. DAG activates protein kinase C which in turn phosphorylates MAP kinase.

MAP kinase activation can be detected by several approaches. One approach is based on an evaluation of the phosphorylation state, either unphosphorylated (inactive) or phosphorylated (active). The phosphorylated protein has a slower mobility in SDS-PAGE and can therefore be compared with the unstimulated protein using Western blotting. Alternatively, antibodies specific for the phosphorylated protein are available (New England Biolabs) which can be used to detect

an increase in the phosphorylated kinase. In either method, cells are stimulated with the test compound and then extracted with Laemmli buffer. The soluble fraction is applied to an SDS-PAGE gel and proteins are transferred electrophoretically to nitrocellulose or Immobilon. Immunoreactive bands are detected by standard Western blotting technique. Visible or chemiluminescent signals are recorded on film and may be quantified by densitometry.

Another approach is based on evaluation of the MAP kinase activity via a phosphorylation assay. Cells are stimulated with the test compound and a soluble extract is prepared. The extract is incubated at 30°C for 10 min with gamma-^{32}P-ATP, an ATP regenerating system, and a specific substrate for MAP kinase such as phosphorylated heat and acid stable protein regulated by insulin, or PHAS-I. The reaction is terminated by the addition of H_3PO_4 and samples are transferred to ice. An aliquot is spotted onto Whatman P81 chromatography paper, which retains the phosphorylated protein. The chromatography paper is washed and counted for ^{32}P in a liquid scintillation counter. Alternatively, the cell extract is incubated with gamma-^{32}P-ATP, an ATP regenerating system, and biotinylated myelin basic protein bound by streptavidin to a filter support. The myelin basic protein is a substrate for activated MAP kinase. The phosphorylation reaction is carried out for 10 min at 30°C. The extract can then by aspirated through the filter, which retains the phosphorylated myelin basic protein. The filter is washed and counted for ^{32}P by liquid scintillation counting.

<u>Cell proliferation assay</u>

Receptor activation of the orphan receptor may lead to a mitogenic or proliferative response which can be monitored via ^{3}H-thymidine uptake. When cultured cells are incubated with ^{3}H-thymidine, the thymidine translocates into the nuclei

where it is phosphorylated to thymidine triphosphate. The nucleotide triphosphate is then incorporated into the cellular DNA at a rate that is proportional to the rate of cell growth. Typically, cells are grown in culture for 1-3 days. Cells are forced into quiescence by the removal of serum for 24 hrs. A mitogenic agent is then added to the media. 24 hrs later, the cells are incubated with ^{3}H-thymidine at specific activities ranging from 1 to 10 µCi/ml for 2-6 hrs. Harvesting procedures may involve trypsinization and trapping of cells by filtration over GF/C filters with or without a prior incubation in TCA to extract soluble thymidine. The filters are processed with scintillant and counted for ^{3}H by liquid scintillation counting. Alternatively, adherent cells are fixed in MeOH or TCA, washed in water, and solubilized in 0.05% deoxycholate/0.1 N NaOH. The soluble extract is transferred to scintillation vials and counted for ^{3}H by liquid scintillation counting.

Alternatively, cell proliferation can be assayed by measuring the expression of an endogenous or heterologous gene product, expressed by the cell line used to transfect the orphan receptor, which can be detected by methods such as, but not limited to, florescence intensity, enzymatic activity, immunoreactivity, DNA hybridization, polymerase chain reaction, etc.

Promiscuous second messenger assays

It is not possible to predict, a priori and based solely upon the GPCR sequence, which of the cell's many different signaling pathways any given orphan receptor will naturally use. It is possible, however, to coax receptors of different functional classes to signal through a pre-selected pathway through the use of promiscuous G_α subunits. For example, by providing a cell based receptor assay system with an endogenously supplied promiscuous G_α subunit such as $G_{\alpha 15}$ or

$G_{\alpha 16}$ or a chimeric G_α subunit such as $G_{\alpha qz}$, a GPCR, which might normally prefer to couple through a specific signaling pathway (e.g., G_s, G_i, G_q, G_0, etc.), can be made to couple through the pathway defined by the promiscuous G_α subunit and upon agonist activation produce the second messenger associated with that subunit's pathway. In the case of $G_{\alpha 15}$, $G_{\alpha 16}$ and/or $G_{\alpha qz}$ this would involve activation of the G_q pathway and production of the second messenger IP_3. Through the use of similar strategies and tools, it is possible to bias receptor signaling through pathways producing other second messengers such as Ca^{++}, cAMP, and K^+ currents, for example (Milligan, 1999).

It follows that the promiscuous interaction of the exogenously supplied G_α subunit with the orphan receptor alleviates the need to carry out a different assay for each possible signaling pathway and increases the chances of detecting a functional signal upon receptor activation.

<u>Methods for recording currents in *Xenopus* oocytes</u>

Oocytes are harvested from *Xenopus laevis* and injected with mRNA transcripts as previously described (Quick and Lester, 1994; Smith et al.,1997). The test receptor of this invention and Gα subunit RNA transcripts are synthesized using the T7 polymerase ("Message Machine," Ambion) from linearized plasmids or PCR products containing the complete coding region of the genes. Oocytes are injected with 10 ng synthetic receptor RNA and incubated for 3-8 days at 17 degrees. Three to eight hours prior to recording, oocytes are injected with 500 pg promiscuous Gα subunits mRNA in order to observe coupling to Ca^{++} activated Cl^- currents. Dual electrode voltage clamp (Axon Instruments Inc.) is performed using 3 M KCl-filled glass microelectrodes having resistances of 1-2 MOhm. Unless otherwise specified, oocytes are voltage clamped at a holding potential of -80 mV. During

recordings, oocytes are bathed in continuously flowing (1-3 ml/min) medium containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.5 (ND96). Drugs are applied either by local perfusion from a 10 µl glass capillary tube fixed at a distance of 0.5 mm from the oocyte, or by switching from a series of gravity fed perfusion lines.

Other oocytes may be injected with a mixture of receptor mRNAs and synthetic mRNA encoding the genes for G-protein-activated inward rectifier channels (GIRK1 and GIRK4, U.S. Patent Nos. 5,734,021 and 5,728,535 or GIRK1 and GIRK2) or any other appropriate combinations (see, e.g., Inanobe et al., 1999). Genes encoding G-protein inwardly rectifying K^+ (GIRK) channels 1, 2 and 4 (GIRK1, GIRK2, and GIRK4) may be obtained by PCR using the published sequences (Kubo et al., 1993; Dascal et al., 1993; Krapivinsky et al., 1995 and 1995b) to derive appropriate 5′ and 3′ primers. Human heart or brain cDNA may be used as template together with appropriate primers.

Heterologous expression of GPCRs in *Xenopus* oocytes has been widely used to determine the identity of signaling pathways activated by agonist stimulation (Gundersen et al., 1983; Takahashi et al., 1987). Activation of the phospholipase C (PLC) pathway is assayed by applying test compound in ND96 solution to oocytes previously injected with mRNA for the mammalian orphan receptor (with or without promiscuous G proteins) and observing inward currents at a holding potential of -80 mV. The appearance of currents that reverse at -25 mV and display other properties of the Ca^{++}-activated Cl^- (chloride) channel is indicative of mammalian receptor-activation of PLC and release of IP3 and intracellular Ca^{++}. Such activity is exhibited by GPCRs that couple to G_q or G_{11}.

Measurement of inwardly rectifying K^+ (potassium) channel

(GIRK) activity may be monitored in oocytes that have been co-injected with mRNAs encoding the mammalian orphan receptor plus GIRK subunits. GIRK gene products co-assemble to form a G-protein activated potassium channel known to be activated (i.e., stimulated) by a number of GPCRs that couple to G_i or G_o (Kubo et al., 1993; Dascal et al., 1993). Oocytes expressing the mammalian orphan receptor plus the GIRK subunits are tested for test compound responsivity by measuring K^+ currents in elevated K^+ solution containing 49 mM K^+.

In the present invention, oocytes were harvested from *Xenopus laevis* and injected with mRNA transcripts as previously described (Quick and Lester, 1994; Smith et al.,1997). SNORF36a RNA transcripts were synthesized using the T7 polymerase ("Message Machine", Ambion) from the plasmid BO109 linearized with NotI. Oocytes were injected with 5-25 ng synthetic RNA and incubated for 3-8 days at 17°C. Dual electrode voltage clamp (Axon Instruments Inc.) was performed using 3 M KCl-filled glass microelectrodes having resistances of 1-2 MOhm. Unless otherwise specified, oocytes were voltage clamped at a holding potential of -80 mV. During recordings, oocytes were bathed in continuously flowing (1-3 ml/min) medium containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.5 (ND96), and the appropriate ligand.

Experiments were carried out under minimal light conditions which required compound weighing, final dilutions and oocyte recordings to be performed under photographic darkroom lights ("Brightlab Junior" safelight). In some cases, the ligands were briefly exposed to room light (5-10 min.) during the weighing procedure. For eliciting physiological responses from oocytes, the light stimulus was a 60 W tungsten lamp set at a distance of 30 cm from the oocyte. Drugs were applied

either by superfusion, switching from a series of gravity fed perfusion lines, or by local perfusion from a 10 µl glass capillary tube fixed at a distance of 0.5 mm from the oocyte. Experiments were carried out at room temperature. All values are expressed as mean ± standard error of the mean.

Membrane preparations

Cell membranes expressing the receptor protein of this invention are useful for certain types of assays including but not restricted to ligand binding assays, GTP-g-S binding assays, and others. The specifics of preparing such cell membranes may in some cases be determined by the nature of the ensuing assay but typically involve harvesting whole cells and disrupting the cell pellet by sonication in ice cold buffer (e.g. 20 mM Tris HCl, mM EDTA, pH 7.4 at 4° C). The resulting crude cell lysate is cleared of cell debris by low speed centrifugation at 200xg for 5 min at 4° C. The cleared supernatant is then centrifuged at 40,000xg for 20 min at 4° C, and the resulting membrane pellet is washed by suspending in ice cold buffer and repeating the high speed centrifugation step. The final washed membrane pellet is resuspended in assay buffer. Protein concentrations are determined by the method of Bradford (1976) using bovine serum albumin as a standard. The membranes may be used immediately or frozen for later use.

Generation of baculovirus

The coding region of DNA encoding the human receptor disclosed herein may be subcloned into pBlueBacIII into existing restriction sites or sites engineered into sequences 5' and 3' to the coding region of the polypeptides. To generate baculovirus, 0.5 µg of viral DNA (BaculoGold) and 3 µg of DNA construct encoding a polypeptide may be co-transfected into 2 x 10^6 *Spodoptera frugiperda* insect Sf9 cells by the calcium phosphate co-precipitation method, as

outlined by Pharmingen (in "Baculovirus Expression Vector System: Procedures and Methods Manual"). The cells then are incubated for 5 days at 27°C.

The supernatant of the co-transfection plate may be collected by centrifugation and the recombinant virus plaque purified. The procedure to infect cells with virus, to prepare stocks of virus and to titer the virus stocks are as described in Pharmingen's manual.

<u>Localization of mRNA coding for human and rat SNORF36</u>

<u>Quantitative RT-PCR using a fluorogenic probe with real time detection</u>: Quantitative RT-PCR using fluorogenic probes and a panel of mRNA extracted from human and total RNA extracted from rat tissue was used to characterize the localization of rat and human SNORF36.

This assay utilizes two oligonucleotides for conventional PCR amplification and a third specific oligonucleotide probe that is labeled with a reporter at the 5' end and a quencher at the 3' end of the oligonucleotide. In the instant invention, FAM (6-carboxyfluorescein) and JOE (6 carboxy-4.5-dichloro-2,7-dimethoxyfluorescein) were the two reporters that were utilized and TAMRA (6-carboxy-4,7,2,7'-tetramethylrhodamine) was the quencher. As amplification progresses, the labeled oligonucleotide probe hybridizes to the gene sequence between the two oligonucleotides used for amplification. The nuclease activity of Taq, or rTth thermostable DNA polymerases is utilized to cleave the labeled probe. This separates the quencher from the reporter and generates a fluorescent signal that is directly proportional to the amount of amplicon generated. This labeled probe confers a high degree of specificity. Non-specific amplification is not detected as the labeled probe does not hybridize. All experiments were conducted in a PE7700 Sequence Detection

System (Perkin Elmer, Foster City CA),

Quantitative RT-PCR: For the detection of RNA encoding SNORF36 receptors, quantitative RT-PCR was performed on mRNA extracted from tissue. Reverse transcription and PCR reactions were carried out in 50 µl volumes using rTth DNA polymerase (Perkin Elmer).

Primers for human SNORF36 were designed to amplify the long subtype selectively (SNORF36b) or to a region common to both SNORF36a and SNORF36b. Amounts of RNA encoding the short subtype (SNORF36a) were calculated by subtracting the amount of SNORF36b from the amount of total SNORF36. Primers with the following sequences were used:

Human SNORF36 subtype non-selective:

Forward primer:
SNORF36b2-457F
5'-GGCTGCGAGTTCTATGCCTT-3' (SEQ ID NO: 36)

Reverse primer
SNORF36b2-547r
5'-TTACCAGGTAGCGGTCCAGG-3' (SEQ ID NO: 37)

Fluorogenic oligonucleotide probe:
SNORF36b2-483T
5'(6-FAM)-AGCTCTCTTTGGCATTTCCTCCATGATCA-(*TAMRA)3'* (SEQ ID NO: 38)

Human SNORF36b (long subtype) selective:

Forward primer:
SNORF36b b sel-256F
5'-CTGGGCAACCTGACGGTC-3' (SEQ ID NO: 39)

Reverse primer
SNORF36b b sel-346R
5′-CAGGTGTCCGGAGGCTTCT-3′ (SEQ ID NO: 40)

Fluorogenic oligonucleotide probe:
SNORF36b b sel-294T
5′(6-FAM)-TGTGCTTCGTGGAGTCACTGTGATGAT-(*TAMRA)3′* (SEQ ID NO: 41)

Rat SNORF36

Forward primer
SNORF36-rat-34F
5′-TCCACTGGCCACCATCG-3′ (SEQ ID NO: 42)

Reverse primer
SNORF36-rat-107R
5′-GGGCATAGAGCCAGACACCTAG-3′ (SEQ ID NO: 43)

Fluorogenic oligonucleotide probe:
SNORF36-rat-52T
5′(6-FAM)-CATGAGATCCAAGAGACGGACGGCA-(*TAMRA)3′* (SEQ ID NO: 44)

Using these primer pairs, amplicon length is 90 bp for human SNORF36-non subtype-selective, 90 bp for human SNORF36b-selective, and 73 bp for rat SNORF36. Each human SNORF36 RT-PCR reaction contained 50 ng mRNA and each rat SNORF36 RT-PCR reaction contained 100 ng total RNA. Oligonuceotide concentrations were: 500 nM of forward and reverse primers, and 200 nM of fluorogenic probe. Concentrations of reagents in each reaction were: 300 μM each of dGTP; dATP; dCTP; 600 μM UTP; 3.0mM Mn(OAc)2 ; 50 mM Bicine; 115 mM potassium acetate, 8% glycerol, 5 units rTth DNA polymerase, and 0.5 units of uracil N-glycosylase. Buffer for RT-PCR reactions also contained a fluor used as a passive reference (ROX:

Perkin Elmer proprietary passive reference I). All reagents for RT-PCR (except mRNA and oligonucleotide primers) were obtained from Perkin Elmer (Foster City, CA). Reactions were carried using the following thermal cycler profile: 50°C 2 min., 60°C 30 min., 95°C 5 min., followed by 40 cycles of: 94°C, 20 sec., 62°C 1 min.

Positive controls for PCR reactions consisted of amplification of the target sequence from a plasmid construct when available. Standard curves for quantitation of human SNORF36 were constructed using the SNORF36 gene in a plasmid construct. RNA extracted from whole brain was used to construct a standard curve for rat SNORF36. Negative controls consisted of mRNA blanks, as well as primer and mRNA blanks. To confirm that the mRNA was not contaminated with genomic DNA, PCR reactions were carried out without reverse transcription using Taq DNA polymerase. Integrity of RNA was assessed by amplification of RNA coding for cyclophilin or glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Following reverse transcription and PCR amplification, data was analyzed using Perkin Elmer sequence detection software. The fluorescent signal from each well was normalized using an internal passive reference, and data was fitted a standard curve to obtain relative quantities of SNORF36 mRNA expression.

Expression of SNORF36 mRNA in human retina

To determine if SNORF36 mRNA is expressed in retina, RT-PCR was performed on human retina Quick Clone cDNA (Clontech, Palo Alto, CA) using two different primer pairs (BB788, a forward SNORF36 primer, with BB789, a reverse SNORF36 primer; or BB796, a forward SNORF36 primer with BB798, a reverse SNORF36 primer). PCR was performed using the Expand Long Template PCR System (Roche Molecular Biochemicals, Indianapolis, IN) under the following conditions: 94°C hold

for 5 minutes; 40 cycles of 94°C for 30 seconds, 68°C for 1.5 minutes; 68°C hold for 7 minutes; 4°C hold until ready for agarose gel electrophoresis. As controls, similar PCR reactions were carried out using human whole brain Quick Clone cDNA, human genomic DNA (Clontech, Palo Alto, CA), or water as templates.

BB788	5'- TCCTCCATGATCACCCTGACGGC -3'	(SEQ ID NO: 45)
BB789	5'- TCTGGAGAGCCCGTCCTGTCTCC -3'	(SEQ ID NO: 46)
BB796	5'- GGCAACCTGACGGTCATCTATACC -3'	(SEQ ID NO: 47)
BB798	5'- TTGGACGCCACACCAAAGGTGGCC -3'	(SEQ ID NO: 48)

Chromosomal localization for human SNORF36 receptor gene

Chromosomal localization for the human SNORF36 receptor gene was established using a panel of radiation hybrids prepared by the Stanford Human Genome Center (SHGC) and distributed by Research Genetics, Inc. The "Stanford G3" panel of 83 radiation hybrids was analyzed by PCR using the same primers, probes and thermal cycler profiles as used for localization. 20 ng of DNA was used in each PCR reaction. Data was submitted to the RH Server (SHGC) which linked the SNORF36 gene sequences to specific markers. NCBI LocusLink and NCBI GeneMap '99 were used to further analyze the data.

RESULTS AND DISCUSSION

Identification of a fragment of the human SNORF36 receptor

A human placental genomic library was screened, under reduced stringency conditions, with oligonucleotide probes directed to the third, fifth and sixth transmembrane regions of the human serotonin 5-HT1_D receptor. Positively-hybridizing clones were isolated, plaque-purified, characterized by Southern blot analysis, and sequenced. One clone, hp10b, contained a 2.3 kb *Hind*III/*Xba*I fragment which hybridized with the human 5-HT1_D-derived oligonucleotide probes and was subsequently subcloned into a pUC vector. This clone, called K39, was a partial gene fragment, encoding TMII and TMIII and a possible downstream intron of a putative novel GPCR.

Isolation of a full-length human SNORF36 receptor

In order to obtain additional sequence for this receptor, a human hippocampal cDNA library was screened by PCR using primers directed against K39. One positive pool was successfully subdivided until a single clone, TL252, was isolated. DNA sequencing of this clone revealed that it contained TMI through TMVII but was lacking the NH_2 and COOH termini. The remaining sequence, including the initiating methionine and the stop codon were obtained by 5′ and 3′ RACE performed on human hippocampal cDNA. The human hippocampal cDNA library was re-screened and two positive clones were identified, 260-13-1 and 143-33-3, which contained TMI through the stop codon. These two clones both had an 11 amino acid insert in the first intracellular loop that was not present in TL252.

A full-length cDNA clone for SNORF36a (without the 11 amino acid insert) was obtained by ligating together a 1021 bp *BamHI*/*Bgl*I fragment from a hippocampal cDNA PCR product along

with a 1180 bp *Bgl*I/*Eco*RI fragment from the human hippocampal library pool 260.13.1 into a *BamH*I/*Eco*RI-cut pcDNA3.1. This construct, BO108, had a single nucleotide mutation which was corrected by site-directed mutagenesis. The corrected construct, BO109, was renamed pcDNA3.1-hSNORF36a-f. Analysis of 9 independent PCR products from human hippocampal cDNA revealed the presence of an allelic variation. Nucleotide 39 (Figures 1A-1C and 3A-3C) is either an adenine or a guanine. A full-length clone for SNORF36b (with the 11 amino acid insert) was obtained by ligating together a 1130 bp *Nsp*I/*Hind*III fragment from a PCR of BO108 along with a 390 bp *BamH*I/*Nsp*I fragment from one of the PCR products described above into a *BamH*I/*Hind*III-cut pcDNA3.1. The resulting SNORF36b construct, BO110, was renamed pcDNA3.1-hSNORF36b-f.

The largest open reading frame in SNORF36a is 1434 nucleotides, which is predicted to encode a protein of 478 amino acids. Using a downstream methionine results in an open frame of 1377 nucleotides and is predicted to encode a protein of 459 amino acids. The nucleotide and amino acid sequences of SNORF36a are shown in Figures 1A-1C and 2A-2B, respectively. The largest open reading frame in SNORF36b is 1467 nucleotides, which is predicted to encode a protein of 489 amino acids. Using a downstream methionine results in an open frame of 1410 nucleotides and is predicted to encode a protein of 470 amino acids. The nucleotide and amino acid sequences of SNORF36b are shown in Figures 3A-3C and 4A-4C respectively. Hydropathy analysis of both SNORF36a and SNORF36b protein are consistent with a putative topography of seven transmembrane domains, indicative of the G protein-coupled receptor family.

A comparison of nucleotide and peptide sequences of human SNORF36a and SNORF36b with sequences contained in the Genbank, EMBL, and SwissProtPlus databases reveals that the

amino acid sequences of these clones are most related to the *Xenopus* melanopsin receptor (45% identity), giant octopus rhodopsin (38% identity), giant scallop rhodopsin (36% identity), Japanese flying squid and cuttlefish rhodopsins (35% identities), squid rhodopsin (34% identity), human peropsin and human RGR (31% identities), human encephalopsin (30% identity), human rhodopsin (28% identity) and bovine rhodopsin (27% identity). There were no sequences in the Genbank databases (Genembl, STS, EST, GSS, or SwissProt) that were identical to SNORF36.

Human SNORF36a and SNORF36b have seven potential protein kinase C (PKC) phosphorylation motifs at serine 111 in the first intracellular loop (numbers in this section are relative to SNORF36b in Figures 4A-4C), serine 194 in the second intracellular loop, threonine 276 in the third intracellular loop, and at serines 386, 395, and 415 and threonine 399 in the carboxy-terminal tail. There is also one potential N-linked glycosylation site at asparagine 88 in the first transmembrane domain and one cAMP phosphorylation site at serine 390. SNORF36a and SNORF36b also have two potential casein kinase II phosphorylation sites at serine 425 and threonine 432 in the carboxy-terminal tail.

Isolation of a Fragment of the Rat Homologue of SNORF36

A fragment of the rat homologue of SNORF36 was amplified from rat genomic DNA by low stringency PCR using oligonucleotide primers designed against the human SNORF36. The sequence of this fragment was then used to generate rat SNORF36 PCR primers which were used to amplify under high stringency a SNORF36 fragment from rat spinal cord cDNA. This fragment, KO56, contains 250 nucleotides of rat SNORF36, from the 3' end of TMII to the middle of TMV. The nucleotide and amino acid sequences of the rat SNORF36 fragment are shown in Figures 5 and 6, respectively. The rat SNORF36 fragment

shares 86% nucleotide and 88% amino acid identities with the human SNORF36 (Figures 7 and 8). KO56 was renamed pEXJ-T3T7-rSNORF36p. There were no sequences in the Genbank databases (Genembl, STS, EST, GSS, or SwissProt) that were identical to rat SNORF36.

Isolation of a full-length rat SNORF36 receptor

In order to obtain a full-length cDNA for rat SNORF36, a rat spinal cord cDNA library was screened by PCR using primers directed against the rat SNORF36 fragment, KO56. One positive pool was successfully subdivided until a single clone, BO132, was isolated. DNA sequencing of this clone revealed that it contained an open reading frame of 1422 nucleotides, 148 nucleotides of 5'UT and approximately 1000 nucleotides of 3'UT. The construct BO132,in the expression vector pEXJ.T7, was renamed pEXJ.T7-rSNORF36-f.

The largest open reading frame in the rat SNORF36 construct BO132 is 1422 nucleotides, which is predicted to encode a protein of 474 amino acids. The nucleotide and amino acid sequences of rat SNORF36 are shown in Figures 9A-9C and 10A-10C, respectively. Hydropathy analysis of the rat SNORF36 protein are consistent with a putative topography of seven transmembrane domains, indicative of the G protein-coupled receptor family.

Rat SNORF36 shares 81% nucleotide identity and 79% amino acid identity with human SNORF36a (Figures 11A-11D and 12A-12B, respectively). A comparison of nucleotide and peptide sequences of rat SNORF36 with sequences contained in the Genbank, EMBL, and SwissProtPlus databases reveals that the amino acid sequences of these clones are most related to the *Xenopus* melanopsin receptor (46% identity), giant octopus rhodopsin and giant scallop rhodopsin (35% identities), cuttlefish rhodopsin (34% identity), Japanese flying squid

and mouse peropsin (33% identities), human RGR (32% identity), human and bovine rhodopsin (28% identities) and human encephalopsin (27% identity). There were no sequences in the Genbank databases (Genembl, STS, EST, GSS, or SwissProt) that were identical to rat SNORF36.

Rat SNORF36 has six potential protein kinase C (PKC) phosphorylation motifs at serine 183 in the second intracellular loop, threonine 265 in the third intracellular loop, and at serines 381 and 385, and threonines 460 and 463 in the carboxy-terminal tail. There are also three potential N-linked glycosylation sites at asparagines 31 and 35 in the amino terminal tail and at asparagine 88 in the first transmembrane domain. There is one cAMP phosphorylation site at threonine 187 in the fourth transmembrane domain, and three potential casein kinase II phosphorylation sites at serines 411 and 452 and threonine 418 in the carboxy-terminal tail.

Ca^{2+} Mobilization and Phosphoinositide Hydrolysis in SNORF36a-expressing Cells

Since SNORF36a is similar to invertebrate opsins, it was hypothesized that it may couple to G_q G-protein and may induce phosphoinositide hydrolysis followed by release of intracellular Ca^{2+}. Therefore, Ca^{2+} mobilization and inositol phosphate (IP) accumulation were evaluated in hSNORF36a-transfected Cos-7 cells. Exposure to laser light of hSNORF36a-transfected Cos-7 cells, kept in dark, in the FLIPR resulted in Ca^{2+} mobilization in the absence of any exogenous ligand (Figure 13A). This observation suggested that an endogenous chromophore, probably a retinal isomer, was already attached to the hSNORF36a receptor, and exposure to laser light resulted in photoisomerization of the ligand and activation of the receptor. Since this situation did not

allow us to study the effect of an exogenously added ligand, we 'photobleached' the hSNORF36a- and vector-transfected Cos-7 cells by exposing them to light for 90-120 minutes prior to the experiment. Photobleaching is a phenomenon where exposure to light results in photoisomerization of the endogenous ligand, eventually leading to release of the ligand and formation of an opsin with an empty binding pocket. This technique is used widely to dissociate the endogenous ligand from rhodopsin. Upon photobleaching, the Ca^{2+} mobilization response to laser light in the hSNORF36a-transfected Cos-7 cells was either reduced or completely abolished (Figure 13B), implying dissociation of the endogenous ligand. Therefore, photobleaching was used for all subsequent experiments unless indicated otherwise.

Opsins can be activated by several retinal analogues, including all-*trans*-retinal (Surya and Knox, 1998) which is a 'natural' agonist formed upon photoisomerization of 11-*cis*-retinal. Upon addition of retinal analogues to photobleached SNORF36a-transfected Cos-7 cells, significant Ca^{2+} mobilization was observed (Figures 14A, 15A). The rank order of potency was 9-*cis*-retinal (EC_{50}:152nM) > all-*trans*-retinal (EC_{50}:263 nM) > 13-*cis*-retinal (EC_{50}:477nM) (See Figure 15A). A similar response to all-*trans*-retinal was seen in photobleached SNORF36a-transfected HEK293 cells (Figure 15B). In contrast, retinals did not change intracellular Ca^{2+} levels in the vector-transfected Cos-7 cells (Figure 14B). Collectively, these results indicate that SNORF36a is an opsin which can be activated by retinals. To confirm these results further, we examined the effect of SNORF36a on IP formation. Concentration-dependent increase in ^{3}H-IP accumulation was seen upon all-*trans*-retinal addition (Figure 16A) in SNORF36a-transfected Cos-7 cells exposed to light but not in vector-transfected cells (Figure 16A). In contrast, in SNORF36a-transfected Cos-7 cells not exposed to light,

all-*trans*-retinal did not change ^{3}H-IP levels, indicating that light may be required either for dissociation of endogenous ligand or activation of the receptor (Figure 16B). Collectively, these results suggest that activation of hSNORF36a can induce phosphoinositide hydrolysis which may be upstream of intracellular Ca^{2+} mobilization. Finally, we tested the potential antagonism by β-ionone which is structurally similar to retinals. β-ionone, at 10μM concentration, significantly antagonized responses to all-*trans*-retinal and 9-*cis*-retinal in hSNORF36a-transfected Cos-7 cells (Figures 17A and 17B). The antagonism by β-ionone appeared to be insurmountable (Figures 17A and 17B). This may be due to the intrinsic characteristics of β-ionone-hSNORF36a interaction. Alternatively, it may represent the hemi-equilibrium conditions achieved in the assay. Nonetheless, these results confirm that retinal-like structure can bind to this receptor. These results are consistent with the notion that SNORF36a is an opsin coupled to Ca^{2+} mobilization and phosphoinositide hydrolysis, most likely via G_q G-protein, and is activated by retinals.

Photic Responses From Oocytes Expressing SNORF36a

The experiments with mammalian cells suggest that SNORF36a, stimulated by the presence of retinoids, can activate a second messenger response that includes release of inositol triphosphates and intracellular free Ca^{++}. Heterologous expression in *Xenopus* oocytes has been widely used to study the ligand binding and functional activity of a variety of G-protein coupled receptors (Gundersen et al., 1983; Takahashi et al., 1987), including rhodopsin (Khorana et al., 1988). Activation of the PLC pathway in oocytes leads to stimulation of the endogenous Ca^{++}-activated Cl^- current. We sought to determine if oocytes expressing SNORF36a could be stimulated by retinoids, and if sensitivity to retinoids was dependent upon activation by light.

Upon acute administration of retinoids to oocytes expressing SNORF36a, no ionic currents were detected under dark conditions or subsequent to the light stimulus. This result suggested that preincubation of ligands may be necessary to see activation of the receptors. To detect light-dependent ionic responses in *Xenopus* oocytes expressing bovine opsin, Khorana and co-workers (1988) incubated oocytes for 30-40 minutes with 11-*cis* retinal to permit the formation of rhodopsin. In another example, the photosensitivity of *Xenopus* melanophores has been seen to occur only following for several hours preincubation with all-*trans* retinal (Rollag, 1996). Following these examples, oocytes expressing SNORF36a were pre-incubated in the dark for 24-48 h with all-*trans*-retinal, 13-*cis*-retinal, or all-*trans*-retinoic acid (ATRA) (each at 100 nM) in $ND96^{++}$ (Figure 19). When preincubated with any of these ligands, the oocytes produced rapidly desensitizing Cl^- currents in response to the light stimulus (Figures 18A and 19). Current amplitudes ranged from 100 to 500 nA, and appeared quite similar in waveform to those elicited by stimulation of receptors that are known to activate PLC (Gundersen et al., 1983). The currents generated by SNORF36a stimulation were considerably larger in amplitude than those reported for oocytes expressing bovine rod opsin (about 5 nA, Khorana et al., 1988). The reason for this is likely due to the different signal transduction pathways activated by these two receptors. SNORF36a may evoke release of intracellular Ca^{++} via activation of $G\alpha_q$, whereas vertebrate opsin couples to transducin and cannot directly activate the PLC pathway. Except for ATRA, the retinoids that were exposed to room light before incubation with the oocytes expressing SNORF36a produced significantly larger current amplitudes (about 5-fold) than non-exposed retinoids (Figures 18B and 19). Control oocytes, not injected with SNORF36a mRNA, that were pre-incubated with all-*trans*-retinal or 13-*cis*-retinal did

not respond to light (Figure 18C; n = 10 oocytes).

Detection of RNA coding for human SNORF36 receptors

mRNA was isolated from multiple tissues (listed in Table 1) and assayed as described.

Human SNORF36

Quantitative RT-PCR using a fluorgenic probe demonstrated mRNA encoding human SNORF36 to be localized in highest abundance in CNS tissue. All CNS tissues assayed demonstrate measurable levels of SNORF36 RNA.

Highest levels are found in the caudate-putamen, amygdala and hippocampus. Localization of high levels of SNORF36 RNA to the caudate-putamen indicates a role in modulation of dopaminergic function, or the modulation of extrapyramidal motor systems. High levels of SNORF36 RNA in the hippocampal formation and amygdala support the hypothesis that SNORF36 is involved in the modulation of learning and memory. It may also have a role in the regulation of fear, mood, and may provide a target for the treatment of depression, anxiety, phobias and mood disorders. Other regions of the CNS containing SNORF36 RNA include the spinal cord and thalamus, implying an important role in sensory transmission or modulation (including nociception). SNORF36 is also expressed in lower levels in the substania nigra, hypothalamus, and cerebellum. The broad distribution of SNORF36 RNA throughout the CNS implies a modulatory role in multiple systems within the CNS.

Fetal brain, although expressing SNORF36 mRNA does so in much lower levels than that found in the adult. In fetal brain, SNORF36 RNA is barely detectable. There is a 70-fold difference in mRNA levels between fetal and adult brain. It is not known at this time if the developmental regulation

occurs in all regions within the CNS or is restricted to selected areas. The time course of this increase has not been examined and would be important in understanding the function of this receptor.

In peripheral tissue, most tissues assayed expressed measurable SNORF36 mRNA levels. The peripheral tissues expressing the highest levels of SNORF36 mRNA are skeletal muscle, heart and small intestine. Levels in these tissues are 10-fold lower than that detected in the highest CNS regions. Presence of SNORF36 mRNA in these areas implies a role in regulation of contractility, perhaps by a common mechanism. Other tissues assayed contain low levels of SNORF36 mRNA as indicated in Table 1.

Both subtypes (SNORF36a and SNORF36b) were assayed in all tissue samples. Primers for human SNORF36 were designed to amplify the long subtype selectively (SNORF36b) or to a region common to both SNORF36a and SNORF36b. Amounts of RNA encoding the short subtype (SNORF36a) were calculated by subtracting the amount of SNORF36b from the amount of total SNORF36. In most regions assayed, SNORF36a is the predominant subtype expressed. The exceptions are the caudate-putamen and fetal lung. In these tissues, SNORF36a accounts for 31% and 43%, respectively, of the total SNORF36. However, in all other tissue assayed, SNORF36a accounts for 60% to 100% of the total SNORF36 present in tissue.

In summary, the distribution of human SNORF36 mRNA implies broad regulatory function in the CNS, most notably in modulation of extrapyramidal motor systems, modulation of the limbic system, and sensory transmission. Its presence, albeit at lower levels, in peripheral tissues implies a broad regulatory role in multiple organ systems. The predominance of SNORF36a in most tissue indicates that functionally, it is the dominant subtype.

Rat SNORF36

As with the human SNORF36 receptor mRNA, all central nervous system structures assayed contain rat SNORF36 RNA. However, the highest levels of rat SNORF36 RNA are found in the retina (Table 2). The retina contains 5-fold more RNA encoding SNORF36 than any other tissue. The high levels found in the retina imply a function in vision or photoentrainment. The hypothesis that rat SNORF36 plays a role in circadian rhythm is supported by the fact that substantial levels of SNORF36 RNA are also expressed in the pineal gland, as well as the hypothalamus (Table 2). The localization and functional data imply that SNORF36 is a non-rod, non-cone, ocular photoreceptor. It has been suggested that non-rod, non-cone ocular photoreceptors are responsible for photoentrainment and regulation of melatonin synthesis in the pineal gland (Freedman et al., 1999, Lucas et al., 1999).

Other regions containing high levels of SNORF36 RNA include trigeminal ganglia, spinal cord, and medulla. Localization to these structures as well as moderate levels in the dorsal root ganglia, strongly suggest a role in sensory transmission (or modulation) including nociceptive stimuli. Localization in the medulla also implies regulation of autonomic centers controlling respiration and cardiovascular function. Other CNS regions containing high levels of SNORF36 RNA include the amygdala, and substanta nigra. High levels in the amygdala and other limbic (or limbic related) structures suggests a role in modulation of mood, fear, phobia, anxiety and may provide a therapeutic target for the treatment of depression and other neuropsychiatric disorders. Localization to the substantia nigra (in conjunction with expression in the striatum) implies a role in regulation of dopaminergic systems, and may provide a therapeutic target for treatment of movement disorders such as Parkinsons disease or tardive dyskinesea.

The high levels of SNORF36 mRNA expressed in the hypothalamus described previously, in addition to the potential role in regulation of circadian rhythm, also indicates a role in neuroendocrine regulation, regulation of appetite and other functions that are modulated by the hypothalamus. High levels in the amygdala suggest a role in modulation of mood, fear, phobia, anxiety and may provide a therapeutic target for the treatment of depression and other neuropsychiatric disorders.

The presence of lower levels of SNORF36 RNA in other areas such as the hippocampal formation, olfactory bulb, cerebral cortex, cerebellum and other areas suggests multiple diverse functions as suggested in Table 2.

Non-neuronal tissue expressing high levels of SNORF36 RNA include the ovary and testes. Levels in these areas are comparable to many CNS regions including the cerebral cortex and cerebellum (Table 2). This strongly suggests a role in endocrine regulation or reproductive function, by neuronal or extraneuronal mechanisms. Other peripheral tissues showing moderate amounts of SNORF36 mRNA are listed in Table 2.

In summary, the high levels or SNORF36 RNA in the retina, pineal gland, and hypothalamus suggest a role as a non-rod, non-cone ocular photoreceptor involved in regulation of circadian rhythms. The distribution of rat SNORF36 mRNA throughout the CNS implies broad regulatory function in the nervous system. Potential functions include modulation of sensory transmission, modulation of extrapyramidal motor systems, and modulation of the limbic system. The ovary and testes are among multiple peripheral organs that may be regulated by SNORF36. Its presence, albeit at lower levels, in other peripheral tissues implies a broad regulatory role in multiple organ systems.

Table 1

Summary of distribution of mRNA coding for human SNORF36 receptors *(long and short forms)*.

Amounts of mRNA encoding human SNORF36 expressed as, mean (whole brain standard curve), % of highest expressing tissue (CPu ± SEM).

Region	SNORF36 % of max	SNORF 36b % of max	SNORF 36a % of max	SNORF 36a % of SNORF36	Potential applications
amygdala	50.10 ±5.73	14.64	35.47	70.78	Depression phobias, anxiety, mood disorders
caudate-putamen	100.00 ±7.89	69.18	30.82	30.82	Modulation of dopamine-rgic function, Modulation of extra-pyramidal motor systems
cerebellum	0.31 ±0.07	not detected	0.31	100	Motor coordination
fetal brain	0.22 ±0.05	0.01	0.22	97.28	Developmental disorders

Region	SNORF36 % of max	SNORF 36b % of max	SNORF 36a % of max	SNORF 36a % of SNORF36	Potential applica-tions
fetal kidney	0.61 ±0.10	not detected	0.61	100	Develop-mental disorders
fetal liver	0.04 ±0.01	0.01	0.03	63.08	Develop-mental disorders
fetal lung	0.42 ±0.11	0.24	0.18	42.79	Develop-mental disorders
heart	7.44 ±2.46	not detected	7.44	100	Cardio-vascular disorders
hippo-campus	26.28 ±1.76	10.52	15.76	59.96	Cognition/ memory
hypo-thalamus	0.77 ±0.07	0.04	0.72	94.49	appetite/ obesity, neuro-endocrine regulation
kidney	0.16 ±0.04	trace	0.16	100	Hyperten-sion, electro-lyte balance
liver	trace	trace	0.02	NA	Diabetes
lung	trace	trace	0.03	NA	Respira-tory disorders, asthma
pancreas	0.07 ±0.01	not detected	0.07	100	Diabetes, endocrine disorders

Region	SNORF36 % of max	SNORF 36b % of max	SNORF 36a % of max	SNORF 36a % of SNORF36	Potential applica-tions
pituitary	4.38 ±0.78	0.17	4.21	96.20	Endocrine/ neuro-endocrine regulation
placenta	0.03 ±0.01	0.05 ±0.02	0.03	100	Gestation-al abnormali-ties
small intestine	6.24 ±1.51	0.34	5.90	94.51	Gastro-intestinal disorders
spinal cord	7.60 ±1.38	0.06	7.54	99.18	Analgesia, sensory modulation and trans-mission
spleen	0.10 ±0.02	trace	0.10	100	Immune disorders
stomach	trace	trace	trace	NA	Gastro-intestinal disorders
skeletal muscle	10.51 ±0.88	0.30	10.21	97.15	Musculo-skeletal disorders
substantia nigra	3.73 ±0.22	0.17	3.57	95.48	Modulation of dopamine-rgic function. Modulation of motor coordina-tion.

Region	SNORF36 % of max	SNORF 36b % of max	SNORF 36a % of max	SNORF 36a % of SNORF36	Potential applica-tions
thalamus	5.56 ±0.70	1.19	4.37	78.62	Sensory integra-tion disorders
whole brain	15.82 ±2.28	4.97	10.85	68.59	

Table 2

Summary of distribution of mRNA coding for rat SNORF36 receptors

mRNA encoding SNORF36 is expressed as % of highest expressing tissue: retina ± SEM.

Tissue	SNORF36	Potential applications
adipose	1.93 ±0.21	metabolic disorders
adrenal cortex	2.62 ±0.48	regulation of steroid hormones
adrenal medulla	2.86 ±0.24	regulation of epinephrine release
amygdala	9.52 ±0.75	depression, phobias, anxiety, mood disorders
aorta	1.08 ±0.07	cardiovascular disorders
celiac plexus	1.58 ±0.14	modulation of autonomic function
cerebellum	4.81 ±0.43	motor coordination
cerebral cortex	6.35 ±0.41	Sensory and motor integration, cognition
choroid plexus	7.02 ±0.93	regulation of cerebrospinal fluid
colon	0.61 ±0.05	gastrointestinal disorders
dorsal root ganglia	4.18 ±0.23	sensory transmission
duodenum	0.86 ±0.10	gastrointestinal disorders
heart	1.79 ±0.19	cardiovascular indications
hippocampus	3.45 ±0.37	cognition/memory
hypothalamus	9.13 ±0.62	appetite/obesity, neuroendocrine regulation

Tissue	SNORF36	Potential applications
kidney	0.95 ±0.07	electrolyte balance, hypertension
liver	trace	diabetes
medulla	9.33 ±0.57	analgesia, motor coordination
nucleus accumbens	4.29 ±0.36	regulation of dopaminergic function, drug addiction, neuropsychiatric disorders
olfactory bulb	3.96 ±0.33	olfaction
ovary	6.83 ±0.66	reproductive function
pancreas	0.30 ±0.04	diabetes, endocrine disorders
pineal	8.37 ±0.21	regulation of melatonin release
pituitary	3.68 ±0.37	endocrine/neuroendocrine regulation
retina	100 ±7.26	visual disorders, circadian rhythms
salivary gland	1.26 ±0.21	
spinal cord	14.47 ±0.44	analgesia, sensory modulation and transmission
spleen	0.27 ±0.14	immune disorders
stomach	0.30 ±0.02	gastrointestinal disorders
striated muscle	2.08 ±0.18	musculoskeletal disorders
striatum	3.72 ±0.67	modulation of dopaminergic function, motor disorders

Tissue	SNORF36	Potential applications
substantia nigra	11.92 ±2.46	modulation of dopaminergic function, modulation of motor coordination
testes	5.48 ±0.21	reproductive function
thalamus	3.85 ±0.16	sensory integration disorders
thymus	3.07 ±0.37	immune disorders
trigeminal ganglia	19.13 ±2.83	sensory transmission
urinary bladder	1.48 ±0.11	urinary incontinence
uterus	1.16 ±0.14	reproductive disorders
vas deferens	2.85 ±0.21	reproductive function
whole brain	4.12 ±0.39	

<u>Expression of SNORF36 mRNA in human retina</u>

To determine if SNORF36 mRNA is expressed in retina, RT-PCR was performed in human retina cDNA using two pairs of SNORF36-specific primers. Single bands of the expected sizes were visualized following agarose gel electrophoresis for each primer pair. Somewhat weaker bands were observed in similar RT-PCR reactions using whole brain cDNA as the template. In contrast, no bands were observed in RT-PCR reactions in which water was used as the template. As both primer pairs were designed to cross introns, similar sized bands were not detected in RT-PCR reactions in which genomic DNA was used as the template. These results demonstrate that SNORF36 mRNA is expressed in human retina.

Chromosomal localization of human SNORF36 receptor genes

The human SNORF36 gene maps to SHGC-57810 and SHGC-57490 which is localized to chromosome 10q22.3.

REFERENCES

Arnheiter, H., "Eyes viewed from the skin" *Nature* 391:632-633 (1998).

Blackshaw, S., and Snyder, S.H., "Parapinopsin, a novel catfish opsin localized to the parapineal organ, defines a new gene family" *J. Neurosci.* 17:8083-8092 (1997).

Blackshaw, S., and Snyder, S.H., "Encephalopsin: a novel mammalian extraretinal opsin discretely localized in the brain" *J. Neurosci.* 19: 3681-3690 (1999).

Bradford, M.M., "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding", *Anal. Biochem.* 72: 248-254 (1976).

Burns, C.C., et al., "Indentification and deletion of sequences required for feline leukemia virus RNA packaging and construction of a high-titer feline leukemia virus packaging cell line", *Virology* 222(1): 14-20 (1996).

Bush, et al., "Nerve growth factor potentiates bradykinin-induced calcium influx and release in PC12 cells" *J. Neurochem.* 57: 562-574(1991).

Campbell, et al., "Light treatment for sleep disorders: consensus report V. Age-related disturbances" *J. Biol. Rhythms* 10: 151-154 (1995).

Chan, T., et al., "Introduction of hydroxyl-bearing amino acids causes bathochromic spectral shifts in rhodopsin. Amino acid substitutions responsible for red-green color pigment spectral tuning" *J. Biol. Chem.* 267: 9478-9480 (1992).

Chu, Y.Y., et al., "Characterization of the rat A2a adenosine receptor gene", *DNA Cell Biol.* 15(4): 329-337 (1996).

Cohen, G.B., et al., "Constitutive activation of opsin: influence of charge at position 134 and size at position 296" *Biochem.* 32: 6111-6115 (1993).

Cooke, K.M., et al., "The effects of evening light exposure on the sleep of elderly women expressing sleep complaints" *J. Behav. Med.* 21: 103-114 (1998).

Dascal, N., et al., "Atrial G protein-activated K^+ channel: expression cloning and molecular properties" *Proc. Natl. Acad. Sci. USA* 90: 10235-10239 (1993).

Fong, T.M., et al., "Mutational analysis of neurokinin receptor function" *Can. J. Physio. Pharmacol.* 73(7): 860-865 (1995).

Foster, R.G., "Shedding light on the biological clock" *Neuron* 20: 829-832 (1998).

Foster, R.G., et al., "Opsin localization and chromophore retinoids identified within the basal brain of the lizard *Anolis carolinensis*" *J. Comp. Physiol. A.* 172: 33-45 (1993).

Foster, R.G., et al., "Identification of vertebrate deep brain photoreceptors" *Neurosci. Biobehav. Rev.* 18: 541-546 (1994).

Franke, H., et al., "An improved low-permeability in vitro-model of the blood-brain barrier: transport studies on retinoids, sucrose, haloperidol, caffeine and mannitol" *Brain Res.* 818: 65-71 (1999).

Freedman, M.S., et al., "Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors" *Science* 284: 502-504 (1999).

Gartner, W., and Towner, P., "Invertebrate visual pigments" *Photochem. Photobiol.* 62: 1-16 (1995).

Grace, M.S., et al., "Light perception in the vertebrate brain: an ultrastructural analysis of opsin- and vasoactive intestinal peptide-immunoactive neurons in Iguanid lizards" *J. Comp. Neurol.* 367: 575-594 (1996).

Graziano, M.P. et al., "The amino terminal domain of the glucagon-like peptide-1 receptor is a critical determinant of subtype specificity" Receptors Channels 4(1): 9-17 (1996).

Guan, X.M., et al., "Determination of Structural Domains for G Protein Coupling and Ligand Binding in β3 - Adrenergic Receptor" *Mol. Pharmacol.* 48(3): 492-498 (1995).

Gundersen, C.B., et al., "Serotonin receptors induced by exogenous messenger RNA in Xenopus oocytes" *Proc. R. Soc. Lond. B. Biol. Sci.* 219(1214): 103-109 (1983).

Han, M., et al., "The effects of amino acid replacements of glycine 121 on transmembrane helix 3 of rhodopsin" *J. Biol. Chem.* 271: 32330-32226 (1996).

Hao, W., and Fong, H.K.W., "The endogenous chromophore of retinal G protein-coupled receptor opsin from the pigment epithelium" *J. Biol. Chem.* 274: 6085-6090 (1999).

Hara-Nishimura, I., et al., "Cloning and nucleotide sequence of cDNA for retinochrome, retinal photoisomerase from the squid retina" *FEBS Lett.* 271: 106-110 (1990).

Hargrave, P.A., and McDowell, J.H., "Rhodopsin and phototransduction: a model system for G protein-linked receptors" *FASEB J* 6: 2323-2331 (1992).

Hu, S., et al., "Unbleachable rhodopsin with an 11-cis-locked eight-membered ring retinal: the visual transduction process", *Biochem.* 33: 408-416 (1994).

Inanobe, A., et al., "Characterization of G-protein-gated K^+ channels composed of Kir3.2 subunits in dopaminergic neurons of the substantia nigra" *J. of Neuroscience* 19(3): 1006-1017 (1999).

Khorana, H.G., et al., "Expression of a bovine rhodopsin gene in Xenopus oocytes: demonstration of light-dependent ionic currents" *Proc. Natl. Acad. Sci. U.S.A.* 85: 7917-7921 (1988).

Kiselev, A., and Subramaniam, S., "Activation and regeneration of rhodopsin in the insect visual cycle" *Science* 266: 1369-1373 (1994).

Kochendoerfer, G.G., et al., "Hoe color visual pigments are tuned" *Trends Biol. Sci.* 24: 300-305 (1999).

Krapivinsky, G., et al., "The cardiac inward rectifier K^+ channel subunit, CIR, does not comprise the ATP-sensitive K^+ channel, IKATP" *J. Biol. Chem.* 270: 28777-28779 (1995b).

Krapivinsky, G., et al., "The G-protein-gated atrial K^+ channel IKACh is a heteromultimer of two inwardly rectifying $K(^+)$-channel proteins" *Nature* 374: 135-141 (1995).

Kubo, Y., et al., "Primary structure and functional expression of a rat G-protein-coupled muscarinic potassium channel" *Nature* 364: 802-806 (1993).

Lack, L., and Wright, H., "The effect of evening bright light in delaying the circadian rhythms and lengthening the sleep of early morning awakening insomniacs" *Sleep* 16: 436-443 (1993).

Lazareno, S. and Birdsall, N.J.M. "Pharmacological characterization of acetylcholine stimulated [35S]-GTPgS binding mediated by human muscarinic m1-m4 receptors: antagonist studies", *Br. J. Pharmacology* 109: 1120-1127 (1993)

Lucas, R.J., et al., "Regulation of the mammalian pineal by non-rod, non-cone, ocular photoreceptors" *Science* 284: 505-507 (1999).

Max, M., et al., "Pineal opsin: a nonvisual opsin expressed in chick pineal" *Science* 267: 1502-1506 (1995).

Max, M., et al., "Light-dependent activation of rod transducin by pineal opsin" *J. Biol. Chem.* 273: 26820-26826 (1998).

Milligan, G., et al., Chimaeric G alpha proteins: their potential use in drug discovery" *Trends Pharmacol. Sci.* 20: 118-124 (1999).

Muller, P.J., and Wilson, B.C., "An update on the penetration depth of 630 nm light in normal and malignant human brain tissue *in vivo*" *Phys. Med. Biol.* 31: 1295-1297 (1986).

Murphy, P. J., and Campbell, S.S., "Enhanced performance in elderly subjects following bright light treatment of sleep maintenance insomnia" *J. Sleep Res.* 5: 165-172 (1996).

Nobes, C., et al., "Activation of the GTP-binding protein Gq

by rhodopsin in squid photoreceptors" *Biochem. J.* 287: 545-548 (1992).

Okano, T., et al., "Pinopsin is a chicken pineal photoreceptive molecule" *Nature* 372: 94-97 (1994).

Oren, D.A., et al., "Treatment of seasonal affective disorder with green light and red light", *Am. J. Psychiatry* 148: 509-511 (1991).

Pardridge, W.M., et al., "Restricted transport of vitamin D and A derivatives through the rat blood-brain barrier" *J. Neurochem.* 44: 1138-1141 (1985).

Partonen, T., "Short note: magnetoreception attributed to the efficacy of light therapy" *Med. Hypotheses* 51: 447-448 (1998).

Provencio, I., et al., "Melanopsin: an opsin in melanophores, brain and eye" *Proc. Natl. Acad. Sci. USA* 95: 340-345 (1998).

Quick, M.W. and Lester, H.A., "Methods for expression of excitability proteins in Xenopus oocytes", *Meth. Neurosci.* 19: 261-279 (1994).

Refinetti, R., "Chronobiology: Business and Health Care" Circadian Physiology: pp. 127-160, CRC Press (1999).

Rollag, M.D., "Amphibian melanophores become photosensitive when treated with retinal" *J. Exp. Zoology* 275: 20-26 (1996).

Rosenthal, N.E., et al., "Phase-shifting effects of bright morning light as treatment for delayed sleep phase syndrome" *Sleep* 13: 354-361 (1990).

Salon, J.A. and Owicki, J.A., "Real-time measurements of receptor activity: Application of microphysiometic techniques to receptor biology" *Methods in Neuroscience* 25: 201-224, Academic Press (1996).

Sanger, F., et al., "DNA sequencing with chain-terminating inhibitors" *Proc. Natl. Acad. Sci. USA* 74: 5463-5467 (1977).

Sedgwick, P.M., "Disorders of the sleep-wake cycle in adults" *Postgrad. Med. J.* 74: 134-138 (1998).

Silver, R., et al., "Coexpression of opsin- and VIP-like-immunoreactivity in CSF-contacting neurons of the avian brain" *Cell Tiss. Res.* 253: 189-198 (1988).

Smith, K.E., et al., "Expression cloning of a rat hypothalamic galanin receptor coupled to phosphoinositide turnover." *J. Biol. Chem.* 272: 24612-24616 (1997).

Soni, B.G., et al., "Novel retinal photoreceptors" *Nature* 394: 27-28 (1998).

Soni, B.G., et al., "A novel and ancient vertebrate opsin" *FEBS Lett.* 407: 279-283 (1997).

Spurney, R.F., et al., "The C-terminus of the thromboxane receptor contributes to coupling and desensitization in a mouse mesangial cell line", *J. Pharmacol. Exp. Ther.* 283(1): 207-215 (1997).

Sun, H., et al., "Peropsin, a novel visual pigment-like protein located in the apical microvilli of the retinal pigment epithelium" *Proc. Natl. Acad. Sci. USA* 94: 9893-9898 (1997).

Surya, A., and Knox, B.E., "Enhancement of opsin activity by all-trans-retinal" *Exp. Eye Res.* 66: 599-603 (1998).

Surya, A., et al., "Transducin activation by the bovine opsin apoprotein" *J. Biol. Chem.* 270: 5024-5031 (1995).

Takahashi, T., et al., "Rat brain serotonin receptors in Xenopus oocytes are coupled by intracellular calcium to endogenous channels." *Proc. Natl. Acad. Sci. USA* 84(14): 5063-5067 (1987).

Takanaka, Y., et al., "Light-dependent expression of pinopsin gene in chicken pineal gland" *J. Neurochem.* 70: 908-913 (1998).

Tanaka, T., et al., "Lipocalin-type prostaglandin D-synthase (beta-trace) is a newly recognized type of retinoid transporter" *J. Biol. Chem.* 272: 15789-15795 (1997).

Tao, L., et al., "Structure and developmental expression of the mouse RGR opsin gene" *Mol. Vision* 4: 25-30 (1998).

Terakita, A., et al., "Selective activation of G-protein subtypes by vertebrate and invertebrate rhodopsins" *FEBS Lett.* 439: 110-114 (1998).

Terman, M., and Terman, J.S., "Bright light therapy: side effects and benefits across the symptom spectrum" *J. Clin. Psychiatry* 60: 799-808 (1999).

Tian, W., et al., "Determinants of alpha-Adrenergic Receptor Activation of G protein: Evidence for a Precoupled Receptor/G protein State." *Molecular Pharmacology* 45: 524-553 (1994).

Underwood, D.J. et al., "Structural model of antagonist and

agonist binding to the angiotensin II, AT1 subtype, G protein coupled receptor", *Chem. Biol.* 1(4): 211-221 (1994).

Wade, P.D., et al., "Mammalian cerebral cortical tissue responds to low-intensity visible light" *Proc. Natl. Acad. Sci. USA* 85: 9322-9326 (1988).

Wood, S.F., et al., "Inositol triphosphate production in squid photoreceptors. Activation by light, aluminium fluoride, and guanine nucleotides" *J. Biol. Chem.* 264: 12970-12976 (1989).

Yarfitz, S., and Hurley, J.B., "Transduction mechanisms of vertebrate and invertebrate photoreceptors" *J. Biol. Chem.* 269: 14329-14332 (1994).

Yoshikawa, T., and Oishi, T., "Extraretinal photoreception and circadian systems in nonmammalian vertebrates" *Comp. Biochem. Physiol.* 119B: 65-72 (1998).

What is claimed is:

1. An isolated nucleic acid encoding a mammalian SNORF36 receptor.

2. The nucleic acid of claim 1, wherein the nucleic acid is DNA.

3. The DNA of claim 2, wherein the DNA is cDNA.

4. The DNA of claim 2, wherein the DNA is genomic DNA.

5. The nucleic acid of claim 1, wherein the nucleic acid is RNA.

6. The nucleic acid of claim 1, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor.

7. The nucleic acid of claim 1, wherein the mammalian SNORF36 receptor is a human SNORF36b receptor.

8. The nucleic acid of claim 6, wherein the human SNORF36a receptor has an amino acid sequence identical to that encoded by the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. PTA-203977).

9. The nucleic acid of claim 7, wherein the human SNORF36b receptor has an amino acid sequence identical to that encoded by the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. PTA-203976).

10. The nucleic acid of claim 1, wherein the mammalian SNORF36 receptor is a rat SNORF36 receptor.

11. The nucleic acid of claim 10, wherein the rat SNORF36 receptor has an amino acid sequence identical to that

encoded by the plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216).

12. The nucleic acid of claim 6, wherein the human SNORF36a receptor has an amino acid sequence identical to the amino acid sequence shown in Figures 2A-2B (SEQ ID NO: 2).

13. The nucleic acid of claim 7, wherein the human SNORF36b receptor has an amino acid sequence identical to the amino acid sequence shown in Figures 4A-4C (SEQ ID NO: 4).

14. The nucleic acid of claim 10, wherein the rat SNORF36 receptor has an amino acid sequence identical to the amino acid sequence shown in Figures 10A-10C (SEQ ID NO: 8).

15. A purified mammalian SNORF36 receptor protein.

16. The purified mammalian SNORF36 receptor protein of claim 15, wherein the SNORF36 receptor protein is a human SNORF36a receptor protein.

17. The purified mammalian SNORF36 receptor protein of claim 15, wherein the SNORF36 receptor protein is a human SNORF36b receptor protein.

18. The purified mammalian SNORF36 receptor protein of claim 15, wherein the SNORF36 receptor protein is a rat SNORF36 receptor protein.

19. A vector comprising the nucleic acid of claim 1.

20. A vector comprising the nucleic acid of claim 6 or claim 7.

21. A vector comprising the nucleic acid of claim 10.

22. A vector of claim 19, 20 or 21 adapted for expression in a cell which comprises the regulatory elements necessary for expression of the nucleic acid in the cell operatively linked to the nucleic acid encoding the receptor so as to permit expression thereof, wherein the cell is a bacterial, amphibian, yeast, insect or mammalian cell.

23. The vector of claim 22, wherein the vector is a baculovirus.

24. The vector of claim 19, wherein the vector is a plasmid.

25. The plasmid of claim 24 designated pcDNA3.1-hSNORF36a-f (ATCC Accession No. PTA-203977)

26. The plasmid of claim 24 designated pcDNA3.1-hSNORF36b-f (ATCC Accession No. PTA-203976).

27. The plasmid of claim 24 designated pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216).

28. A cell comprising the vector of claim 22.

29. A cell of claim 28, wherein the cell is a non-mammalian cell.

30. A cell of claim 29, wherein the non-mammalian cell is a *Xenopus* oocyte cell or a *Xenopus* melanophore cell.

31. A cell of claim 28, wherein the cell is a mammalian cell.

32. A mammalian cell of claim 31, wherein the cell is a COS-7 cell, a 293 human embryonic kidney cell, a NIH-3T3 cell, a LM(tk-) cell, a mouse Y1 cell, or a CHO cell.

33. A cell of claim 28, wherein the cell is an insect cell.

34. An insect cell of claim 33, wherein the insect cell is an Sf9 cell, an Sf21 cell or a Trichoplusia ni 5B-4 cell.

35. A membrane preparation isolated from the cell of any one of claims 28, 29, 31, 32, 33 or 34.

36. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within one of the two strands of the nucleic acid encoding the human SNORF36a receptor contained in plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. PTA-203977).

37. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within one of the two strands of the nucleic acid encoding the human SNORF36b receptor contained in plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. PTA-203976).

38. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a

unique sequence present within one of the two strands of the nucleic acid encoding the mammalian SNORF36 receptor contained in plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216).

39. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figures 1A-1C (SEQ ID NO: 1) or (b) the reverse complement thereof.

40. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figures 3A-3C (SEQ ID NO: 3) or (b) the reverse complement thereof.

41. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figures 9A-9C (SEQ ID NO: 7) or (b) the reverse complement thereof.

42. The nucleic acid probe of claim 39, 40 or 41, wherein the nucleic acid is DNA.

43. The nucleic acid probe of claim 39, 40 or 41, wherein the nucleic acid is RNA.

44. An antisense oligonucleotide having a sequence capable

of specifically hybridizing to the RNA of claim 5, so as to prevent translation of the RNA.

45. An antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA of claim 4, so as to prevent transcription of the genomic DNA.

46. An antisense oligonucleotide of claim 44 or 45, wherein the oligonucleotide comprises chemically modified nucleotides or nucleotide analogues.

47. An antibody capable of binding to a mammalian SNORF36 receptor encoded by the nucleic acid of claim 1.

48. An antibody of claim 47, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

49. An agent capable of competitively inhibiting the binding of the antibody of claim 47 to a mammalian SNORF36 receptor.

50. An antibody of claim 47, wherein the antibody is a monoclonal antibody or antisera.

51. A pharmaceutical composition comprising (a) an amount of the oligonucleotide of claim 44 capable of passing through a cell membrane and effective to reduce expression of a mammalian SNORF36 receptor and (b) a pharmaceutically acceptable carrier capable of passing through the cell membrane.

52. A pharmaceutical composition of claim 51, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

53. A pharmaceutical composition of claim 52, wherein the substance which inactivates mRNA is a ribozyme.

54. A pharmaceutical composition of claim 52, wherein the pharmaceutically acceptable carrier comprises a structure which binds to a mammalian SNORF36 receptor on a cell capable of being taken up by the cells after binding to the structure.

55. A pharmaceutical composition of claim 54, wherein the pharmaceutically acceptable carrier is capable of binding to a mammalian SNORF36 receptor which is specific for a selected cell type.

56. A pharmaceutical composition which comprises an amount of the antibody of claim 47 effective to block binding of a ligand to a human SNORF36 receptor and a pharmaceutically acceptable carrier.

57. A transgenic, nonhuman mammal expressing DNA encoding a mammalian SNORF36 receptor of claim 1.

58. A transgenic, nonhuman mammal comprising a homologous recombination knockout of the native mammalian SNORF36 receptor.

59. A transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to the DNA encoding a mammalian SNORF36 receptor of claim 1 so placed within the genome as to be transcribed into antisense mRNA which is complementary to and hybridizes with mRNA encoding the mammalian SNORF36 receptor so as to thereby reduce translation of such mRNA and expression of such receptor.

60. The transgenic, nonhuman mammal of claim 57 or 58,

wherein the DNA encoding the mammalian SNORF36 receptor additionally comprises an inducible promoter.

61. The transgenic, nonhuman mammal of claim 57 or 58, wherein the DNA encoding the mammalian SNORF36 receptor additionally comprises tissue specific regulatory elements.

62. A transgenic, nonhuman mammal of claim 57, 58, or 59, wherein the transgenic, nonhuman mammal is a mouse.

63. A process for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises contacting cells containing DNA encoding, and expressing on their cell surface, the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor.

64. A process for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises contacting a membrane preparation from cells containing DNA encoding, and expressing on their cell surface, the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor.

65. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor.

66. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor has substantially the same amino acid sequence as the human SNORF36a receptor encoded by plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. PTA-203977).

67. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor has substantially the same amino acid sequence as the human SNORF36b receptor encoded by the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. PTA-203976).

68. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor has substantially the same amino acid sequence as that shown in Figures 2A-2B (SEQ ID NO: 2) or Figures 4A-4C (SEQ ID NO: 4).

69. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor has the amino acid sequence shown in Figures 2A-2B (SEQ ID NO: 2) or Figures 4A-4C (SEQ ID NO: 4).

70. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor is a rat SNORF36 receptor.

71. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor has substantially the same amino acid sequence as the rat SNORF36 receptor encoded by plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216).

72. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor has substantially the same amino acid sequence as that shown in Figures 10A-10C (SEQ ID NO: 8).

73. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor has the same amino acid sequence as that shown in Figures 10A-10C (SEQ ID NO: 8).

74. The process of claim 63 or 64, wherein the compound is not previously known to bind to a mammalian SNORF36 receptor.

75. A compound identified by the process of claim 74.

76. A process of claim 63 or 64, wherein the cell is an insect cell.

77. The process of claim 63 or 64, wherein the cell is a mammalian cell.

78. The process of claim 77, wherein the cell is nonneuronal in origin.

79. The process of claim 78, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

80. A process of claim 77, wherein the compound is a compound not previously known to bind to a mammalian SNORF36 receptor.

81. A compound identified by the process of claim 80.

82. A process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises separately contacting cells expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with

both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF36 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF36 receptor.

83. A process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises separately contacting a membrane preparation from cells expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF36 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF36 receptor.

84. A process of claim 82 or 83, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor.

85. A process of claim 82 or 83, wherein the mammalian SNORF36 receptor is a rat SNORF36 receptor.

86. The process of claim 82 or 83, wherein the cell is an insect cell.

87. The process of claim 82 or 83, wherein the cell is a mammalian cell.

88. The process of claim 87, wherein the cell is nonneuronal in origin.

89. The process of claim 88, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

90. The process of claim 89, wherein the compound is not previously known to bind to a mammalian SNORF36 receptor.

91. A compound identified by the process of claim 90.

92. A method of screening a plurality of chemical compounds not known to bind to a mammalian SNORF36 receptor to identify a compound which specifically binds to the mammalian SNORF36 receptor, which comprises

(a) contacting cells transfected with, and expressing, DNA encoding the mammalian SNORF36 receptor with a compound known to bind specifically to the mammalian SNORF36 receptor;

(b) contacting the cells of step (a) with the plurality of compounds not known to bind specifically to the mammalian SNORF36 receptor, under conditions permitting binding of compounds known to bind to the mammalian SNORF36 receptor;

(c) determining whether the binding of the compound known to bind to the mammalian SNORF36 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

(d) separately determining the binding to the mammalian SNORF36 receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF36 receptor.

93. A method of screening a plurality of chemical compounds not known to bind to a mammalian SNORF36 receptor to identify a compound which specifically binds to the mammalian SNORF36 receptor, which comprises

(a) contacting a membrane preparation from cells transfected with, and expressing, DNA encoding the mammalian SNORF36 receptor with the plurality of compounds not known to bind specifically to the mammalian SNORF36 receptor under conditions permitting binding of compounds known to bind to the mammalian SNORF36 receptor;

(b) determining whether the binding of a compound known to bind to the mammalian SNORF36 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

(c) separately determining the binding to the mammalian SNORF36 receptor of each compound

included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF36 receptor.

94. A method of claim 92 or 93, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor.

95. A method of claim 92 or 93, wherein the mammalian SNORF36 receptor is a rat SNORF36 receptor.

96. A method of claim 92 or 93, wherein the cell is a mammalian cell.

97. A method of claim 96, wherein the mammalian cell is non-neuronal in origin.

98. The method of claim 97, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell, a CHO cell, a mouse Y1 cell, or an NIH-3T3 cell.

99. A method of detecting expression of a mammalian SNORF36 receptor by detecting the presence of mRNA coding for the mammalian SNORF36 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the nucleic acid probe of claim 36, 37, 38, 39, 40 or 41 under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the mammalian SNORF36 receptor by the cell.

100. A method of detecting the presence of a mammalian SNORF36 receptor on the surface of a cell which comprises contacting the cell with the antibody of

claim 47 under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of the mammalian SNORF36 receptor on the surface of the cell.

101. A method of determining the physiological effects of varying levels of activity of mammalian SNORF36 receptors which comprises producing a transgenic, nonhuman mammal of claim 57 whose levels of mammalian SNORF36 receptor activity are varied by use of an inducible promoter which regulates mammalian SNORF36 receptor expression.

102. A method of determining the physiological effects of varying levels of activity of mammalian SNORF36 receptors which comprises producing a panel of transgenic, nonhuman mammals of claim 57 each expressing a different amount of mammalian SNORF36 receptor.

103. A method for identifying an antagonist capable of alleviating an abnormality wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor comprising administering a compound to the transgenic, nonhuman mammal of claim 57, 58, or 59, and determining whether the compound alleviates any physiological and/or behavioral abnormality displayed by the transgenic, nonhuman mammal as a result of overactivity of a mammalian SNORF36 receptor, the alleviation of such an abnormality identifying the compound as an antagonist.

104. The method of claim 103, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

105. An antagonist identified by the method of claim 103.

106. A composition comprising an antagonist of claim 105 and a carrier.

107. A method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition of claim 106, thereby treating the abnormality.

108. A method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor comprising administering a compound to the transgenic, nonhuman mammal of claim 57, 58, or 59, and determining whether the compound alleviates any physiological and/or behavioral abnormality displayed by the transgenic, nonhuman mammal, the alleviation of such an abnormality identifying the compound as an agonist.

109. The method of claim 108, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

110. An agonist identified by the method of claim 109.

111. A composition comprising an agonist identified by the method of claim 110 and a carrier.

112. A method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject an effective

amount of the composition of claim 111 so as to thereby treat the abnormality.

113. A method for diagnosing a predisposition to a disorder associated with the activity of a specific mammalian allele which comprises:

(a) obtaining DNA of subjects suffering from the disorder;

(b) performing a restriction digest of the DNA with a panel of restriction enzymes;

(c) electrophoretically separating the resulting DNA fragments on a sizing gel;

(d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a mammalian SNORF36 receptor and labeled with a detectable marker;

(e) detecting labeled bands which have hybridized to the DNA encoding a mammalian SNORF36 receptor of claim 1 to create a unique band pattern specific to the DNA of subjects suffering from the disorder;

(f) repeating steps (a)-(e) with DNA obtained for diagnosis from subjects not yet suffering from the disorder; and

(g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step (e) with the band pattern from step (f) for subjects not yet suffering from the disorder so as

to determine whether the patterns are the same or different and thereby diagnose predisposition to the disorder if the patterns are the same.

114. The method of claim 113, wherein a disorder associated with the activity of a specific mammalian allele is diagnosed.

115. A method of preparing the purified mammalian SNORF36 receptor of claim 15 which comprises:

(a) culturing cells which express the mammalian SNORF36 receptor;

(b) recovering the mammalian SNORF36 receptor from the cells; and

(c) purifying the mammalian SNORF36 receptor so recovered.

116. A method of preparing the purified mammalian SNORF36 receptor of claim 15 which comprises:

(a) inserting a nucleic acid encoding the mammalian SNORF36 receptor into a suitable expression vector;

(b) introducing the resulting vector into a suitable host cell;

(c) placing the resulting host cell in suitable conditions permitting the production of the mammalian SNORF36 receptor;

(d) recovering the mammalian SNORF36 receptor so produced; and optionally

(e) isolating and/or purifying the mammalian SNORF36 receptor so recovered.

117. A process for determining whether a chemical compound is a mammalian SNORF36 receptor agonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF36 receptor with the compound under conditions permitting the activation of the mammalian SNORF36 receptor, and detecting any increase in mammalian SNORF36 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF36 receptor agonist.

118. A process for determining whether a chemical compound is a mammalian SNORF36 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF36 receptor with the compound in the presence of a known mammalian SNORF36 receptor agonist, under conditions permitting the activation of the mammalian SNORF36 receptor, and detecting any decrease in mammalian SNORF36 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF36 receptor antagonist.

119. A process of claim 117 or 118, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

120. A composition which comprises an amount of a mammalian SNORF36 receptor agonist determined by the process of claim 117 effective to increase activity of a mammalian SNORF36 receptor and a carrier.

121. A composition of claim 120, wherein the mammalian SNORF36 receptor agonist is not previously known.

122. A composition which comprises an amount of a mammalian SNORF36 receptor antagonist determined by the process of claim 118 effective to reduce activity of a mammalian SNORF36 receptor and a carrier.

123. A composition of claim 122, wherein the mammalian SNORF36 receptor antagonist is not previously known.

124. A process for determining whether a chemical compound specifically binds to and activates a mammalian SNORF36 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the chemical compound under conditions suitable for activation of the mammalian SNORF36 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian SNORF36 receptor.

125. The process of claim 124, wherein the second messenger response comprises chloride channel activation and the change in second messenger is an increase in the level of chloride current.

126. The process of claim 124, wherein the second messenger response comprises intracellular calcium levels and the change in second messenger is an increase in the measure of intracellular calcium.

127. The process of claim 124, wherein the second messenger response comprises release of inositol phosphate and the change in second messenger is an increase in the level of inositol phosphate.

128. A process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian SNORF36 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with both the chemical compound and a second chemical compound known to activate the mammalian SNORF36 receptor, and with only the second chemical compound, under conditions suitable for activation of the mammalian SNORF36 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian SNORF36 receptor.

129. The process of claim 128, wherein the second messenger response comprises chloride channel activation and the change in second messenger response is a smaller increase in the level of chloride current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

130. The process of claim 129, wherein the second messenger response comprises change in intracellular calcium levels and the change in second messenger response is a smaller increase in the measure of intracellular calcium in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

131. The process of claim 128, wherein the second messenger response comprises release of inositol phosphate and the change in second messenger response is a smaller increase in the level of inositol phosphate in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

132. A process of any of claims 124, 125, 126, 127, 128, 129, 130, or 131, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

133. The process of any one of claims 121, 125, 126, 127, 128, 129, 130, or 131, wherein the cell is an insect cell.

134. The process of any one of claims 124, 125, 126, 127, 128, 129, 130, or 131, wherein the cell is a mammalian cell.

135. The process of claim 134, wherein the mammalian cell is nonneuronal in origin.

136. The process of claim 135, wherein the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

137. The process of claim 124, 125, 126, 127, 128, 129, 130, or 131, wherein the compound is not previously known to bind to a mammalian SNORF36 receptor.

138. A compound determined by the process of claim 137.

139. A composition which comprises an amount of a mammalian SNORF36 receptor agonist determined to be such by the

process of claim 124, 125, 126, or 127, effective to increase activity of the mammalian SNORF36 receptor and a carrier.

140. A composition of claim 139, wherein the mammalian SNORF36 receptor agonist is not previously known.

141. A composition which comprises an amount of a mammalian SNORF36 receptor antagonist determined to be such by the process of claim 128, 129, 130, or 131, effective to reduce activity of the mammalian SNORF36 receptor and a carrier.

142. A composition of claim 141, wherein the mammalian SNORF36 receptor antagonist is not previously known.

143. A method of screening a plurality of chemical compounds not known to activate a mammalian SNORF36 receptor to identify a compound which activates the mammalian SNORF36 receptor which comprises:

(a) contacting cells transfected with and expressing the mammalian SNORF36 receptor with the plurality of compounds not known to activate the mammalian SNORF36 receptor, under conditions permitting activation of the mammalian SNORF36 receptor;

(b) determining whether the activity of the mammalian SNORF36 receptor is increased in the presence of one or more of the compounds; and if so

(c) separately determining whether the activation of the mammalian SNORF36 receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian SNORF36 receptor.

144. A method of claim 143, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

145. A method of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian SNORF36 receptor to identify a compound which inhibits the activation of the mammalian SNORF36 receptor, which comprises:

(a) contacting cells transfected with and expressing the mammalian SNORF36 receptor with the plurality of compounds in the presence of a known mammalian SNORF36 receptor agonist, under conditions permitting activation of the mammalian SNORF36 receptor;

(b) determining whether the extent or amount of activation of the mammalian SNORF36 receptor is reduced in the presence of one or more of the compounds, relative to the extent or amount of activation of the mammalian SNORF36 receptor in the absence of such one or more compounds; and if so

(c) separately determining whether each such compound inhibits activation of the mammalian SNORF36 receptor for each compound included in the plurality of compounds, so as to thereby identify any compound included in such plurality of compounds which inhibits the activation of the mammalian SNORF36 receptor.

146. A method of claim 145, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or rat SNORF36 receptor.

147. A method of any one of claims 143, 144, 145 or 146, wherein the cell is a mammalian cell.

148. A method of claim 147, wherein the mammalian cell is non-neuronal in origin.

149. The method of claim 148, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell or an NIH-3T3 cell.

150. A composition comprising a compound identified by the method of claim 143 or 144 in an amount effective to increase mammalian SNORF36 receptor activity and a carrier.

151. A composition comprising a compound identified by the method of claim 145 or 146 in an amount effective to decrease mammalian SNORF36 receptor activity and a carrier.

152. A method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject a compound which is a mammalian SNORF36 receptor agonist in an amount effective to treat the abnormality.

153. A method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject a compound which is a mammalian SNORF36 receptor antagonist in an amount effective to treat the abnormality.

154. A process for making a composition of matter which specifically binds to a mammalian SNORF36 receptor

which comprises identifying a chemical compound using the process of any one of claims 63, 64, 82, 83, 92 or 93 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

155. The process of claims 154, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

156. A process for making a composition of matter which specifically binds to a mammalian SNORF36 receptor which comprises identifying a chemical compound using the process of any of claims 117, 124, or 143 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

157. The process of claim 156, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

158. A process for making a composition of matter which specifically binds to a mammalian SNORF36 receptor which comprises identifying a chemical compound using the process of any of claims 118, 128 or 145 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

159. The process of claim 158, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

160. A process for preparing a composition which comprises admixing a carrier and a pharmaceutically effective amount of a chemical compound identified by the process of any of claims 63, 64, 82, 83, 92 or 93 or a novel

structural and functional analog or homolog thereof.

161. The process of claim 160, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

162. A process for preparing a composition which comprises admixing a carrier and a pharmaceutically effective amount of a chemical compound identified by the process of any of claims 117, 124, or 143 or a novel structural and functional analog or homolog thereof.

163. The process of claim 162, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

164. A process for preparing a composition which comprises admixing a carrier and a pharmaceutically effective amount of a chemical compound identified by the process of any of claims 118, 128 or 145 or a novel structural and functional analog or homolog thereof.

165. The process of claim 164, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

FIGURE 1A

1	CAACTCAGGATGAACCCTCCTTCGGGGCCAAGAGTCCCGCCCAGCCCAACCCAAGAGCCC	60
61	AGCTGCATGGCCACCCCAGCACCACCCAGCTGGTGGACAGCTCCCAGAGCAGCATCTCC	120
121	AGCCTGGGCCGGCTTCCATCCATCAGTCCCACAGCACCTGGGACTTGGGCTGCTGCCTGG	180
181	GTCCCCCTCCCCACGGTTGA..GTTCCAGACCATGCCCACTATACCCTGGCACAGTGATC	240
241	TTGCTGGTGGGACTCACGGGGATGCTGGGCAACCTGACGGTCATCTATACCTTCTGCAGG	300
301	AGCAGAAGCCTCCGGACACCTGCCAACATGTTCATTATCAACCTCGCGGTCAGCGACTTC	360
361	CTCATGTCCTTCACCCAGGCCCCTGTCTTCTTCACCAGTAGCCTCTATAAGCAGTGGCTC	420
421	TTTGGGGAGACAGGCTGCGAGTTCTATGCCTTCTGTGGAGCTCTCTTTGGCATTTCCTCC	480
481	ATGATCACCCTGACGGCCATCGCCCTGGACCGCTACCTGGTAATCACACGCCCGCTGGCC	540
541	ACCTTTGGTGTGGCGTCCAAGAGGCGTGCGGCATTTGTCCTGCTGGGCGTTTGGCTCTAT	600
601	GCCCTGGCCTGGAGTCTGCCACCCTTCTTCGGCTGGAGCGCCTACGTGCCCGAGGGGTTG	660
661	CTGACATCCTGCTCCTGGGACTACATGAGCTTCACGCCGGCCGTGCGTGCCTACACCATG	720

FIGURE 1B

```
721  CTTCTCTGCTGCTTCGTGTTCTTCCTCCCTCTGCTTATCATCATCTACTGCTACATCTTC  780
781  ATCTTCAGGGCCATCCGGGAGACAGGACGGGCTCTCCAGACCTTCGGGGCCTGCAAGGGC  840
841  AATGGCGAGTCCCTGTGGCAGCGGCAGCGGCTGCAGAGCGAGTGCAAGATGGCCAAGATC  900
901  ATGCTGCTGGTCATCCTCCTCTTCGTGCTCTCCTGGGCTCCCTATTCCGCTGTGGCCCTG  960
961  GTGGCCTTTGCTGGGTACGCACACGTCCTGACACCCTACATGAGCTCGGTGCCAGCCGTC 1020
1021 ATCGCCAAGGCCTCTGCAATCCACAACCCCATCATTTACGCCATCACCCACCCCAAGTAC 1080
1081 AGGGTGGCCATTGCCCAGCACCTGCCCTGCCTGGGGGTGCTGCTGGGTGTATCACGCCGG 1140
1141 CACAGTCGCCCCTACCCCAGCTACCGCTCCACCCACCGCTCCACGCTGACCAGCCACACC 1200
1201 TCCAACCTCAGCTGGATCTCCATACGGAGGCGCCAGGAGTCCCTGGGCTCGGAGAGTGAG 1260
1261 GTGGGCTGGACACACATGGAGGCAGCAGCTGTGTGGGGAGCTGCCCAGCAAGCAAATGGG 1320
1321 CGGTCCCTCTACGGTCAGGGTCTGGAGGACTTGGAAGCCAAGGCACCCCCCAGACCCCAG 1380
1381 GGACACGAAGCAGAGACTCCAGGGAAGACCAAGGGGCTGATCCCCAGCCAGGACCCCAGG 1440
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FIGURE 1C

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1441  ATGTAGGACGCCCACTGGCTCTCCCTTTCTTCTGAGACACATCCAGCCCCCCCACGTCTC 1500

1501  CCTCATAT                                                     1508
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FIGURE 2A

1	M	N	P	P	S	G	P	R	V	P	P	S	P	T	Q	E	P	S	C	M	20
21	A	T	P	A	P	P	S	W	W	D	S	S	Q	S	S	I	S	S	L	G	40
41	R	L	P	S	I	S	P	T	A	P	G	T	W	A	A	A	W	V	P	L	60
61	P	T	V	D	V	P	D	H	A	H	Y	T	L	G	T	V	I	L	L	V	80
81	G	L	T	G	M	L	G	N	L	T	V	I	Y	T	F	C	R	S	R	S	100
101	L	R	T	P	A	N	M	F	I	I	N	L	A	V	S	D	F	L	M	S	120
121	F	T	Q	A	P	V	F	F	T	S	S	L	Y	K	Q	W	L	F	G	E	140
141	T	G	C	E	F	Y	A	F	C	G	A	L	F	G	I	S	S	M	I	T	160
161	L	T	A	I	A	L	D	R	Y	L	V	I	T	R	P	L	A	T	F	G	180
181	V	A	S	K	R	R	A	A	F	V	L	L	G	V	W	L	Y	A	L	A	200
201	W	S	L	P	P	F	F	G	W	S	A	Y	V	P	E	G	L	L	T	S	220
221	C	S	W	D	Y	M	S	F	T	P	A	V	R	A	Y	T	M	L	L	C	240

FIGURE 2B

241 C F V F F L P L L I I I Y C Y I F I F R 260

261 A I R E T G R A L Q T F G A C K G N G E 280

281 S L W Q R Q R L Q S E C K M A K I M L L 300

301 V I L L F V L S W A P Y S A V A L V A F 320

321 A G Y A H V L T P Y M S S V P A V I A K 340

341 A S A I H N P I I Y A I T H P K Y R V A 360

361 I A Q H L P C L G V L L G V S R R H S R 380

381 P Y P S Y R S T H R S T L T S H T S N L 400

401 S W I S I R R R Q E S L G S E S E V G W 420

421 T H M E A A A V W G A A Q Q A N G R S L 440

441 Y G Q G L E D L E A K A P P R P Q G H E 460

461 A E T P G K T K G L I P S Q D P R M 478

FIGURE 3A

```
1    CAACTCAGGATGAACCCTCCTTCGGGGCCAAGAGTCCCGCCCAGCCCAACCCAAGAGCCC   60
61   AGCTGCATGGCCACCCCAGCACCACCCAGCTGGTGGGACAGCTCCCAGAGCAGCATCTCC  120
121  AGCCTGGGCCGGCTTCCATCCATCAGTCCCACAGCACCTGGGACTTGGGCTGCTGCCTGG  180
181  GTCCCCCTCCCCACGGTTGATGTTCCAGACCATGCCCACTATACCCTGGGCACAGTGATC  240
241  TTGCTGGTGGGACTCACGGGGATGCTGGCAACCTGACGGTCATCTATACCTTCTGCAGA  300
301  GCTGTGCTTCGTGGAGTCACTGTGATGATGCAGAGCAGAAGCCTCCGGACACCTGCCAAC  360
361  ATGTTCATTATCAACCTCGCGGTCAGCGACTTCCTCATGTCCTTCACCCAGGCCCCTGTC  420
421  TTCTTCACCAGTAGCCTCTATAAGCAGTGGCTCTTTGGGGAGACAGGCTGCGAGTTCTAT  480
481  GCCTTCTGTGGAGCTCTCTTTGGCATTTCCTCCATGATCACCCTGACGGCCATCGCCCTG  540
541  GACCGCTACCTGGTAATCACACGCCCGCTGGCCACCTTTGGTGTGGCGTCCAAGAGGCGT  600
601  GCGGCATTTGTCCTGCTGGGCGTTTGGCTCTATGCCCTGGCCTGGAGTCTGCCACCCTTC  660
661  TTCGGCTGGAGCGCCTACGTGCCCGAGGGGTTGCTGACATCCTGCTCCTGGGACTACATG  720
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FIGURE 3B

```
721   AGCTTCACGCCGGCCGTGCGTGCCTACACCATGCTTCTCTGCTGCTTCGTGTTCTTCCTC   780
781   CCTCTGCTTATCATCATCTACTGCTACATCTTCATCTTCAGGGCCATCCGGGAGACAGGA   840
841   CGGGCTCTCCAGACCTTCGGGGCCTGCAAGGGCAATGGCGAGTCCCTGTGGCAGCGGCAG   900
901   CGGCTGCAGAGCGAGTGCAAGATGGCCAAGATCATGCTGCTGGTCATCCTCCTCTTCGTG   960
961   CTCTCCTGGGCTCCCTATTCCGCTGTGGCCCTGGTGGCCTTTGCTGGGTACGCACACGTC   1020
1021  CTGACACCCTACATGAGCTCGGTGCCAGCCGTCATCGCCAAGGCCTCTGCAATCCACAAC   1080
1081  CCCATCATTTACGCCATCACCCACCCCAAGTACAGGGTGGCCATTGCCCAGCACCTGCCC   1140
1141  TGCCTGGGGGTGCTGCTGGGTGTATCACGCCGGCACAGTCGCCCCTACCCCAGCTACCGC   1200
1201  TCCACCCACCGCTCCACGCTGACCAGCCACACCTCCAACCTCAGCTGGATCTCCATACGG   1260
1261  AGGCGCCAGGAGTCCCTGGGCTCGGAGAGTGAGGTGGGCTGGACACACATGGAGGCAGCA   1320
1321  GCTGTGTGGGGAGCTGCCCAGCAAGCAAATGGGCGGTCCCTCTACGGTCAGGGTCTGGAG   1380
1381  GACTTGGAAGCCAAGGCACCCCCCAGACCCCAGGGACACGAAGCAGAGACTCCAGGGAAG   1440
```

FIGURE3C

1441 ACCAAGGGGCTGATCCCCAGCCAGGACCCCAGGATGTAGGACGCCCACTGGCTCTCCCTT 1500

1501 TCTTCTGAGACACATCCAGCCCCCCCACGTCTCCCTCATAT 1541

FIGURE 4A

```
1    M N P P S G P R V P P S P T Q E P S C M   20
21   A T P A P P S W W D S S Q S S I S S L G   40
41   R L P S I S P T A P G T W A A A W V P L   60
61   P T V D V P D H A H Y T L G T V I L L V   80
81   G L T G M L G N L T V I Y T F C R A V L   100
101  R G V T V M M Q S R S L R T P A N M F I   120
121  I N L A V S D F L M S F T Q A P V F F T   140
141  S S L Y K Q W L F G E T G C E F Y A F C   160
161  G A L F G I S S M I T L T A I A L D R Y   180
181  L V I T R P L A T F G V A S K R R A A F   200
201  V L L G V W L Y A L A W S L P P F F G W   220
221  S A Y V P E G L L T S C S W D Y M S F T   240
```

FIGURE 4B

241	P	A	V	R	A	Y	T	M	L	L	C	C	F	V	F	F	L	P	L	L	260
261	I	I	I	Y	C	Y	I	F	I	F	R	A	I	R	E	T	G	R	A	L	280
281	Q	T	F	G	A	C	K	G	N	G	E	S	L	W	Q	R	Q	R	L	Q	300
301	S	E	C	K	M	A	K	I	M	L	L	V	I	L	L	F	V	L	S	W	320
321	A	P	Y	S	A	V	A	L	V	A	F	A	G	Y	A	H	V	L	T	P	340
341	Y	M	S	S	V	P	A	V	I	A	K	A	S	A	I	H	N	P	I	I	360
361	Y	A	I	T	H	P	K	Y	R	V	A	I	A	Q	H	L	P	C	L	G	380
381	V	L	L	G	V	S	R	R	H	S	R	P	Y	P	S	Y	R	S	T	H	400
401	R	S	T	L	T	S	H	T	S	N	L	S	W	I	S	I	R	R	R	Q	420
421	E	S	L	G	S	E	S	E	V	G	W	T	H	M	E	A	A	A	V	W	440
441	G	A	A	Q	Q	A	N	G	R	S	L	Y	G	Q	G	L	E	D	L	E	460
461	A	K	A	P	P	R	P	Q	G	H	E	A	E	T	P	G	K	T	K	G	480

FIGURE 4C

481 L I P S Q D P R M 489

FIGURE 5

```
1    CATAGCCATGGACCGCTATCTGGTGATCACACGTCCACTGGCCACCATCGGCATGAGATC   60
61   CAAGAGACGGACGGCACTAGTCCTGCTAGGTGTCTGGCTCTATGCCCTGGCCTGGAGTCT   120
121  GCCGCCTTTCTTTGGCTGGAGCGCCTACGTGCCCGAGGGGCTGCTGACATCCTGCTCCTG   180
181  GGACTACGTGACCTTCACGCCCCTCGTGCGCGCCTACACCATGCTGCTCTTCTGCTTTGT   240
241  CTTCTTCCTC                                                     250
```

FIGURE 6

1	I	A	M	D	R	Y	L	V	I	T	R	P	L	A	T	I	G	M	R	S	20
21	K	R	R	T	A	L	V	L	L	G	V	W	L	Y	A	L	A	W	S	L	40
41	P	P	F	F	G	W	S	A	Y	V	P	E	G	L	L	T	S	C	S	W	60
61	D	Y	V	T	F	T	P	L	V	R	A	Y	T	M	L	L	F	C	F	V	80
81	F	F	L																		83

NOT FURNISHED UPON FILING

FIGURE 7B

```
Rat SNORF36   163 GCTGACATCCTGCTCCTGGGACTACGTGACCTTCACGCCCCTCGTGCGCG 212
                  ||||||||||||||||||||||||| ||| |||||||||   |||||| |
Hum SNORF36   651 GCTGACATCCTGCTCCTGGGACTACATGAGCTTCACGCCGGCCGTGCGTG 700

Rat SNORF36   213 CCTACACCATGCTGCTCTTCTGCTTTGTCTTCTTCCTC............ 250
                  ||||||||||||| |||| |||||| || |||||||||
Hum SNORF36   701 CCTACACCATGCTTCTCTGCTGCTTCGTGTTCTTCCTCCCTCTGCTTATC 750
```

FIGURE 8

```
Rat SNORF36    1 .............IAMDRYLVITRPLATIGMRSKRRTALVLLGVWLYALA  37
                              ||:|||||||||||| |. |||| | |||||||||||
Hum SNORF36  151 ALFGISSMITLTAIALDRYLVITRPLATFGVASKRRAAFVLLGVWLYALA 200

Rat SNORF36   38 WSLPPFFGWSAYVPEGLLTSCSWDYVTFTPLVRAYTMLLFCFVFFL....  83
                 |||||||||||||||||||||||||..||| |||||||| ||||||
Hum SNORF36  201 WSLPPFFGWSAYVPEGLLTSCSWDYMSFTPAVRAYTMLLCCFVFFLPLLI 250
```

FIGURE 9A

```
  1  TTTAAGTCCTCCAAGAGCCTGAGCATGAACTCTCCTTCAGAATCAAGAGTCCCTTCAAGC   60
 61  TTAACTCAGGATCCCAGCTTTACCGCCAGCCCTGCCCTCCTACAAGGCATTTGGAACAGC  120
121  ACTCAGAACATCTCCGTCAGAGTCCAGCTTCTATCCGTTAGCCCCACGACACCTGGGCTT  180
181  CAGGCTGCTGCCTGGGTCCCCTTCCCCACAGTCGACGTCCCAGATCATGCTCACTATACC  240
241  CTAGGCACGGTGATCCTGCTGGTGGGACTCACAGGGATGCTGGGTAACCTGACAGTCATC  300
301  TACACCTTCTGCAGGAATAGAGGCCTGCGGACACCGGCAAACATGCTCATCATCAACCTG  360
361  GCAGTCAGCGACTTCCTTATGTCGTTCACTCAGGCCCCGGTCTTCTTTGCCAGCAGCCTC  420
421  TACAAGAAGTGGCTCTTCGGGGAGACAGGTTGCAAGTTCTATGCCTTCTGTGGGGCTGTC  480
481  TTTGGCATCGTTTCCATGATCACCCTGACAGCCATAGCCATGGACCGCTATCTGGTGATC  540
541  ACACGTCCACTGGCCACCATCGGCATGAGATCCAAGAGACGGACGGCACTAGTCCTGCTA  600
601  GGTGTCTGGCTCTATGCCCTGGCCTGGAGTCTGCCGCCTTTCTTTGGCTGGAGCGCCTAC  660
```

FIGURE 9B

```
661   GTGCCCGAGGGGCTGCTGACATCCTGCTCCTGGGACTACGTGACCTTCACGCCCCTCGTG   720
721   CGCGCCTACACCATGCTGCTCTTCTGCTTTGTCTTCTTCCTCCCTCTGCTCATTATCATC   780
781   TTCTGCTACATCTTCATCTTCAGGGCCATTCGAGAGACAGGCCGGGCCTGTGAGGGCTGT   840
841   GGTGAGTCCCCTCTGCGGCGGCGGCAGTGGCAGCGGCTACAGAGTGAATGGAAGATGGCC   900
901   AAGGTCGCACTGATCGTCATTCTCCTCTTTGTGCTGTCCTGGGCTCCCTACTCCACTGTG   960
961   GCCCTGGTGGGCTTTGCTGGGTACTCGCACATCCTGACGCCCTACATGAGCTCGGTGCCA  1020
1021  GCCGTCATTGCCAAGGCCTCGGCCATCCACAATCCTATCATCTATGCCATCACTCACCCC  1080
1081  AAGTACAGGGCGGCCATTGCTCAGCACTTGCCTTGCCTTGGGGTGCTTCTTGGAGTATCA  1140
1141  GGCCAGCGCAGCCACCCCTCCCTCAGCTACCGCTCTACCCATCGCTCCACACTGAGCAGC  1200
1201  CAGTCCTCAGACCTCAGCTGGATCTCTGGGCAGAAGCGCCAAGAGTCCCTGGGTTCTGAG  1260
1261  AGTGAAGTGGGCTGGACAGACACAGAAACAACAGCTGCGTGGGGAGCTGCCCAGCAAGCA  1320
```

FIGURE 9C

1321 AGTGGACAATCCTTCTGCAGTCATGACCTGGAAGATGGAGAAGTCAAGGCTCCTTCCAGC 1380

1381 CCCCAGGAACAGAAATCCAAGACTCCCAAGACCAAGAGACACCTCCCCAGTCTGGACCGA 1440

1441 AGGATG<u>TAG</u>GATGCCCAGTCCCGTCCCCTCCCT 1473

FIGURE 10A

1	M	N	S	P	S	E	S	R	V	P	S	S	L	T	Q	D	P	S	F	T	20
21	A	S	P	A	L	L	Q	G	I	W	N	S	T	Q	N	I	S	V	R	V	40
41	Q	L	L	S	V	S	P	T	T	P	G	L	Q	A	A	A	W	V	P	F	60
61	P	T	V	D	V	P	D	H	A	H	Y	T	L	G	T	V	I	L	L	V	80
81	G	L	T	G	M	L	G	N	L	T	V	I	Y	T	F	C	R	N	R	G	100
101	L	R	T	P	A	N	M	L	I	I	N	L	A	V	S	D	F	L	M	S	120
121	F	T	Q	A	P	V	F	F	A	S	S	L	Y	K	K	W	L	F	G	E	140
141	T	G	C	K	F	Y	A	F	C	G	A	V	F	G	I	V	S	M	I	T	160
161	L	T	A	I	A	M	D	R	Y	L	V	I	T	R	P	L	A	T	I	G	180
181	M	R	S	K	R	R	T	A	L	V	L	L	G	V	W	L	Y	A	L	A	200
201	W	S	L	P	P	F	F	G	W	S	A	Y	V	P	E	G	L	L	T	S	220

FIGURE 10B

221	C	S	W	D	Y	V	T	F	T	P	L	V	R	A	Y	T	M	L	L	F	240
241	C	F	V	F	F	L	P	L	L	I	I	I	F	C	Y	I	F	I	F	R	260
261	A	I	R	E	T	G	R	A	C	E	G	C	G	E	S	P	L	R	R	R	280
281	Q	W	Q	R	L	Q	S	E	W	K	M	A	K	V	A	L	I	V	I	L	300
301	L	F	V	L	S	W	A	P	Y	S	T	V	A	L	V	G	F	A	G	Y	320
321	S	H	I	L	T	P	Y	M	S	S	V	P	A	V	I	A	K	A	S	A	340
341	I	H	N	P	I	I	Y	A	I	T	H	P	K	Y	R	A	A	I	A	Q	360
361	H	L	P	C	L	G	V	L	L	G	V	S	G	Q	R	S	H	P	S	L	380
381	S	Y	R	S	T	H	R	S	T	L	S	S	Q	S	S	D	L	S	W	I	400
401	S	G	Q	K	R	Q	E	S	L	G	S	E	S	E	V	G	W	T	D	T	420
421	E	T	T	A	A	W	G	A	A	Q	Q	A	S	G	Q	S	F	C	S	H	440

FIGURE 10C

```
441   D L E D G E V K A P S S P Q E Q K S K T   460
461   P K T K R H L P S L D R R M               474
```

FIGURE 11A

```
hSNORF36a    1 ATGAACCCTCCTTCGGGGCCAAGAGTCCCGCCCAGCCCAACCCAAGAGCC 50
               |||||| ||||||| |   ||||||||||  | |||  ||| || || ||
rSNORF36     1 ATGAACTCTCCTTCAGAATCAAGAGTCCCTTCAAGCTTAACTCAGGATCC 50

hSNORF36a   51 CAGCTGCATGGCCACCCCAGCACCACCCAGCTGGTGGGACAGCTCCCAGA 100
               |||||  |  |||| ||| || |  |      |       | |  |    
rSNORF36    51 CAGCTTTACCGCCAGCCCTGCCCTCCTACAAGGCATTTGGAACAGCACTC 100

hSNORF36a  101 GCAGCATCTCCAGCCTGGGCCGGCTTCCATCCATCAGTCCCACAGCACCT 150
                 | |||||||  |   | || ||||| |||| | || |||  |||||
rSNORF36   101 AGAACATCTCCGTCAGAGTCCAGCTTCTATCCGTTAGCCCCACGACACCT 150

hSNORF36a  151 GGGACTTGGGCTGCTGCCTGGGTCCCCCTCCCCACGGTTGATGTTCCAGA 200
               |||  |  |||||||||||||||||| |||||||| || || || |||||
rSNORF36   151 GGGCTTCAGGCTGCTGCCTGGGTCCCCTTCCCCACAGTCGACGTCCCAGA 200

hSNORF36a  201 CCATGCCCACTATACCCTGGGCACAGTGATCTTGCTGGTGGGACTCACGG 250
                ||||| ||||||||||| |||| ||||||| |||||||||||||||| |
rSNORF36   201 TCATGCTCACTATACCCTAGGCACGGTGATCCTGCTGGTGGGACTCACAG 250

hSNORF36a  251 GGATGCTGGGCAACCTGACGGTCATCTATACCTTCTGCAGGAGCAGAAGC 300
               |||||||||| ||||||||  |||||||| ||||||||||||||  ||| ||
rSNORF36   251 GGATGCTGGGTAACCTGACAGTCATCTACACCTTCTGCAGGAATAGAGGC 300

hSNORF36a  301 CTCCGGACACCTGCCAACATGTTCATTATCAACCTCGCGGTCAGCGACTT 350
               || ||||||||| || ||||||| |||| |||||||| || |||||||||||
rSNORF36   301 CTGCGGACACCGGCAAACATGCTCATCATCAACCTGGCAGTCAGCGACTT 350

hSNORF36a  351 CCTCATGTCCTTCACCCAGGCCCCTGTCTTCTTCACCAGTAGCCTCTATA 400
               ||| ||||| ||||| ||||||||| |||||||||  |||| |||||||| |
rSNORF36   351 CCTTATGTCGTTCACTCAGGCCCCGGTCTTCTTTGCCAGCAGCCTCTACA 400
```

FIGURE 11B

```
hSNORF36a   401 AGCAGTGGCTCTTTGGGGAGACAGGCTGCGAGTTCTATGCCTTCTGTGGA 450
                || |||||||||| ||||||||||| ||| |||||||||||||||||||
rSNORF36    401 AGAAGTGGCTCTTCGGGGAGACAGGTTGCAAGTTCTATGCCTTCTGTGGG 450

hSNORF36a   451 GCTCTCTTTGGCATTTCCTCCATGATCACCCTGACGGCCATCGCCCTGGA 500
                ||| ||||||||||    ||||||||||||||||| ||||| ||| ||||
rSNORF36    451 GCTGTCTTTGGCATCGTTTCCATGATCACCCTGACAGCCATAGCCATGGA 500

hSNORF36a   501 CCGCTACCTGGTAATCACACGCCCGCTGGCCACCTTTGGTGTGGCGTCCA 550
                |||||| ||||| |||||||| || ||||||||| | ||  ||   ||||
rSNORF36    501 CCGCTATCTGGTGATCACACGTCCACTGGCCACCATCGGCATGAGATCCA 550

hSNORF36a   551 AGAGGCGTGCGGCATTTGTCCTGCTGGGCGTTTGGCTCTATGCCCTGGCC 600
                |||| ||  ||||| | |||||||| || || ||||||||||||||||||
rSNORF36    551 AGAGACGGACGGCACTAGTCCTGCTAGGTGTCTGGCTCTATGCCCTGGCC 600

hSNORF36a   601 TGGAGTCTGCCACCCTTCTTCGGCTGGAGCGCCTACGTGCCCGAGGGGTT 650
                ||||||||||| || ||||| ||||||||||||||||||||||||||| |
rSNORF36    601 TGGAGTCTGCCGCCTTTCTTTGGCTGGAGCGCCTACGTGCCCGAGGGGCT 650

hSNORF36a   651 GCTGACATCCTGCTCCTGGGACTACATGAGCTTCACGCCGGCCGTGCGTG 700
                ||||||||||||||||||||||||| ||| |||||||||   |||||| |
rSNORF36    651 GCTGACATCCTGCTCCTGGGACTACGTGACCTTCACGCCCCTCGTGCGCG 700

hSNORF36a   701 CCTACACCATGCTTCTCTGCTGCTTCGTGTTCTTCCTCCCTCTGCTTATC 750
                ||||||||||||| |||| |||||| || ||||||||||||||||| ||
rSNORF36    701 CCTACACCATGCTGCTCTTCTGCTTTGTCTTCTTCCTCCCTCTGCTCATT 750

hSNORF36a   751 ATCATCTACTGCTACATCTTCATCTTCAGGGCCATCCGGGAGACAGGACG 800
                ||||||| ||||||||||||||||||||||||||| || |||||||| ||
rSNORF36    751 ATCATCTTCTGCTACATCTTCATCTTCAGGGCCATTCGAGAGACAGGCCG 800
```

FIGURE 11C

```
hSNORF36a   801 GGCTCTCCAGACCTTCGGGGCCTGCAAGGGCAATGGCGAGTCCCTGTGGC 850
rSNORF36    801 GG.........CCTGTGAGGGCTGTGGTGAGTCCCCTCTGCGGCGGCGGC 841

hSNORF36a   851 AGCGGCAGCGGCTGCAGAGCGAGTGCAAGATGGCCAAGATCATGCTGCTG 900
rSNORF36    842 AGTGGCAGCGGCTACAGAGTGAATGGAAGATGGCCAAGGTCGCACTGATC 891

hSNORF36a   901 GTCATCCTCCTCTTCGTGCTCTCCTGGGCTCCCTATTCCGCTGTGGCCCT 950
rSNORF36    892 GTCATTCTCCTCTTTGTGCTGTCCTGGGCTCCCTACTCCACTGTGGCCCT 941

hSNORF36a   951 GGTGGCCTTTGCTGGGTACGCACACGTCCTGACACCCTACATGAGCTCGG 1000
rSNORF36    942 GGTGGGCTTTGCTGGGTACTCGCACATCCTGACGCCCTACATGAGCTCGG 991

hSNORF36a  1001 TGCCAGCCGTCATCGCCAAGGCCTCTGCAATCCACAACCCCATCATTTAC 1050
rSNORF36    992 TGCCAGCCGTCATTGCCAAGGCCTCGGCCATCCACAATCCTATCATCTAT 1041

hSNORF36a  1051 GCCATCACCCACCCCAAGTACAGGGTGGCCATTGCCCAGCACCTGCCCTG 1100
rSNORF36   1042 GCCATCACTCACCCCAAGTACAGGGCGGCCATTGCTCAGCACTTGCCTTG 1091

hSNORF36a  1101 CCTGGGGGTGCTGCTGGGTGTATCACGCCGGCACAGTCGCCCCTACCCCA 1150
rSNORF36   1092 CCTTGGGGTGCTTCTTGGAGTATCAGGCCAGCGCAGCCACCCCTCCCTCA 1141

hSNORF36a  1151 GCTACCGCTCCACCCACCGCTCCACGCTGACCAGCCACACCTCCAACCTC 1200
rSNORF36   1142 GCTACCGCTCTACCCATCGCTCCACACTGAGCAGCCAGTCCTCAGACCTC 1191
```

FIGURE 11D

```
hSNORF36a  1201 AGCTGGATCTCCATACGGAGGCGCCAGGAGTCCCTGGGCTCGGAGAGTGA 1250
rSNORF36   1192 AGCTGGATCTCTGGGCAGAAGCGCCAAGAGTCCCTGGGTTCTGAGAGTGA 1241

hSNORF36a  1251 GGTGGGCTGGACACACATGGAGGCAGCAGCTGTGTGGGGAGCTGCCCAGC 1300
rSNORF36   1242 AGTGGGCTGGACAGACACAGAAACAACAGCTGCGTGGGGAGCTGCCCAGC 1291

hSNORF36a  1301 AAGCAAATGGGCGGTCCCTCTACGGTCAGGGTCTGGAGGACTTGGAAGCC 1350
rSNORF36   1292 AAGCAAGTGGACAATCCTTCTGCAGTCATGACCTGGAAGATGGAGAAGTC 1341

hSNORF36a  1351 AAGGCACCCCCCAGACCCCAGGGACACGAAGCAGAGACTCCAGGGAAGAC 1400
rSNORF36   1342 AAGGCTCCTTCCAGCCCCCAGGAACAGAAATCCAAGACTCC...CAAGAC 1388

hSNORF36a  1401 CAAGGGGCTGATCCCCAGCCAGGACCCCAGGATGTAG              1437
rSNORF36   1389 CAAGAGACACCTCCCCAGTCTGGACCGAAGGATGTAG              1425
```

FIGURE 12A

```
hSNORF36a    1 MNPPSGPRVPPSPTQEPSCMATPA.PPSWWDSSQSSISSLGRLPSISPTA 49
rSNORF36     1 MNSPSESRVPSSLTQDPSFTASPALLQGIWNSTQ.NISVRVQLLSVSPTT 49

hSNORF36a   50 PGTWAAAWVPLPTVDVPDHAHYTLGTVILLVGLTGMLGNLTVIYTFCRSR 99
rSNORF36    50 PGLQAAAWVPFPTVDVPDHAHYTLGTVILLVGLTGMLGNLTVIYTFCRNR 99

hSNORF36a  100 SLRTPANMFIINLAVSDFLMSFTQAPVFFTSSLYKQWLFGETGCEFYAFC 149
rSNORF36   100 GLRTPANMLIINLAVSDFLMSFTQAPVFFASSLYKKWLFGETGCKFYAFC 149

hSNORF36a  150 GALFGISSMITLTAIALDRYLVITRPLATFGVASKRRAAFVLLGVWLYAL 199
rSNORF36   150 GAVFGIVSMITLTAIAMDRYLVITRPLATIGMRSKRRTALVLLGVWLYAL 199

hSNORF36a  200 AWSLPPFFGWSAYVPEGLLTSCSWDYMSFTPAVRAYTMLLCCFVFFLPLL 249
rSNORF36   200 AWSLPPFFGWSAYVPEGLLTSCSWDYVTFTPLVRAYTMLLFCFVFFLPLL 249

hSNORF36a  250 IIIYCYIFIFRAIRETGRALQTFGACKGNGESLWQR...QRLQSECKMAK 296
rSNORF36   250 IIIFCYIFIFRAIRETGR......ACEGCGESPLRRRQWQRLQSEWKMAK 293

hSNORF36a  297 IMLLVILLFVLSWAPYSAVALVAFAGYAHVLTPYMSSVPAVIAKASAIHN 346
rSNORF36   294 VALIVILLFVLSWAPYSTVALVGFAGYSHILTPYMSSVPAVIAKASAIHN 343

hSNORF36a  347 PIIYAITHPKYRVAIAQHLPCLGVLLGVSRRHSRPYPSYRSTHRSTLTSH 396
rSNORF36   344 PIIYAITHPKYRAAIAQHLPCLGVLLGVSGQRSHPSLSYRSTHRSTLSSQ 393
```

FIGURE 12B

```
hSNORF36a    397 TSNLSWISIRRRQESLGSESEVGWTHMEAAAVWGAAQQANGRSLYGQGLE 446
                 .|.||||| .:||||||||||||||  |  |  |||||||.|.|     ||
rSNORF36     394 SSDLSWISGQKRQESLGSESEVGWTDTETTAAWGAAQQASGQSFCSHDLE 443

hSNORF36a    447 DLEAKAPPRPQGHEAETPGKTKGLIPSQDPRM                   478
                 | | |||  ||  ...|| |||  :|| | ||
rSNORF36     444 DGEVKAPSSPQEQKSKTP.KTKRHLPSLDRRM                   474
```

FIGURE 13A

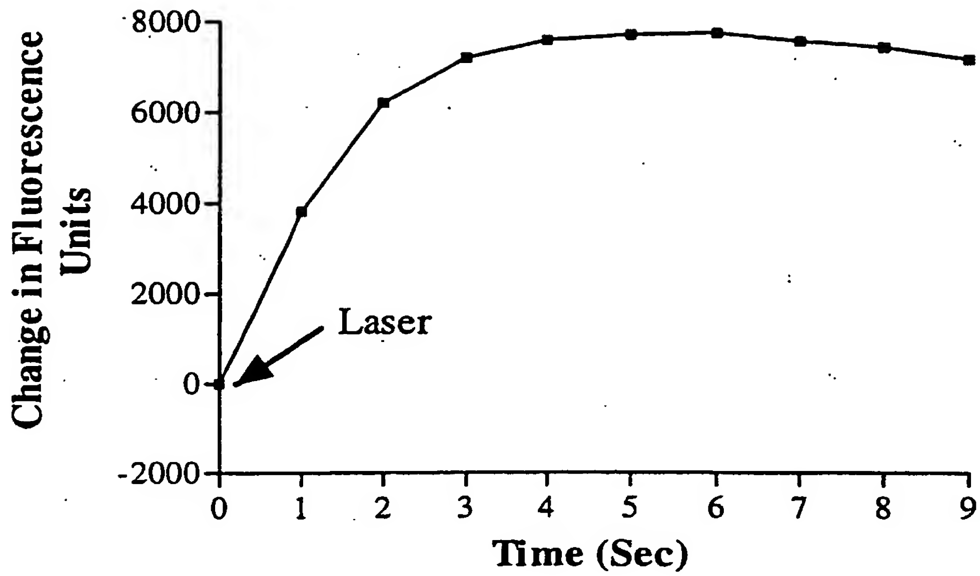


FIGURE 13B

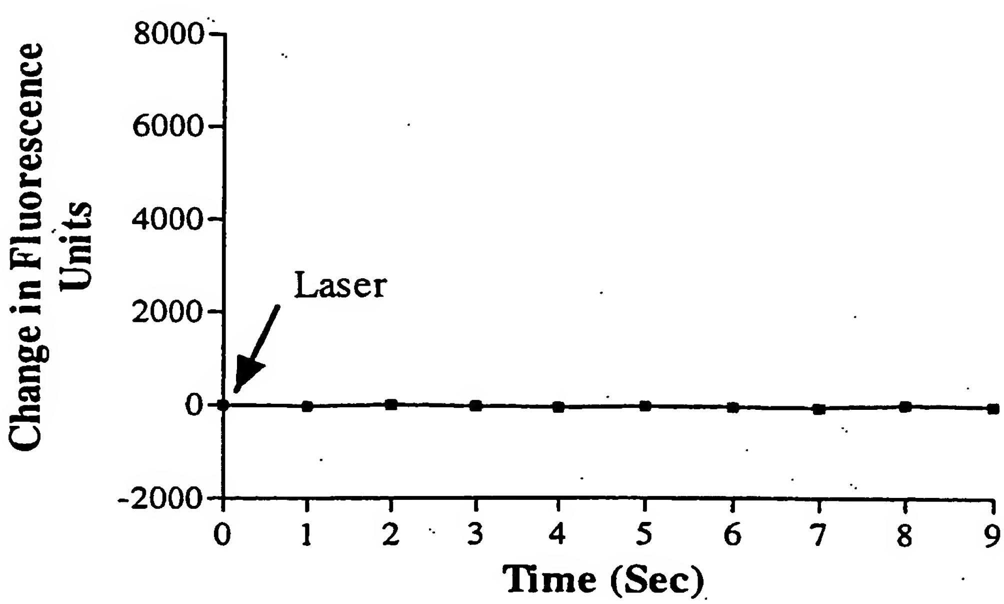


FIGURE 14A

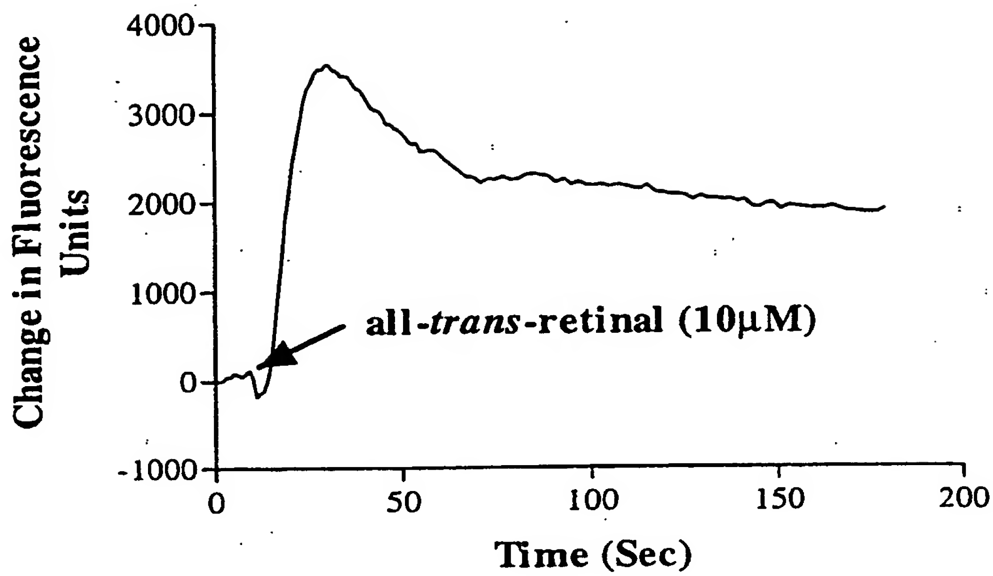


FIGURE 14B

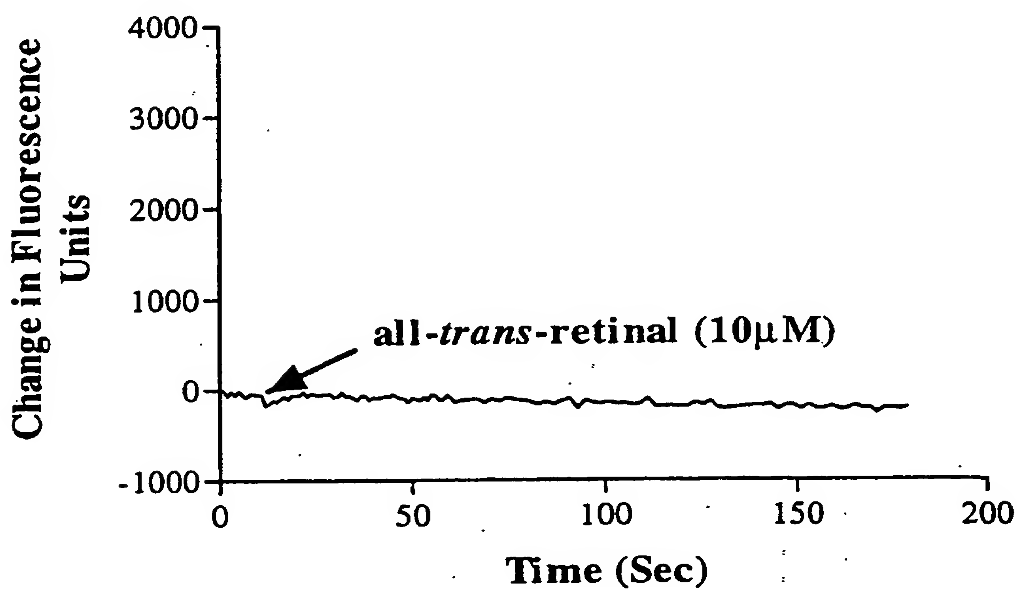


FIGURE 15A

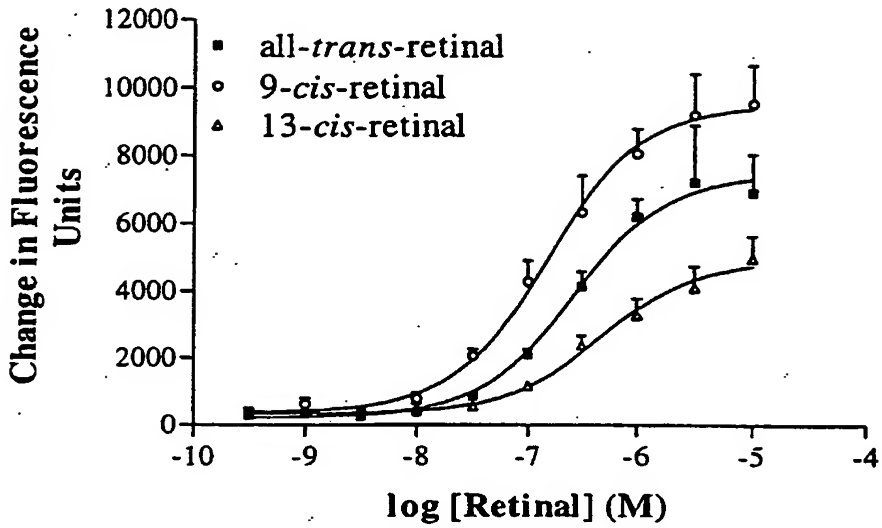


FIGURE 15B

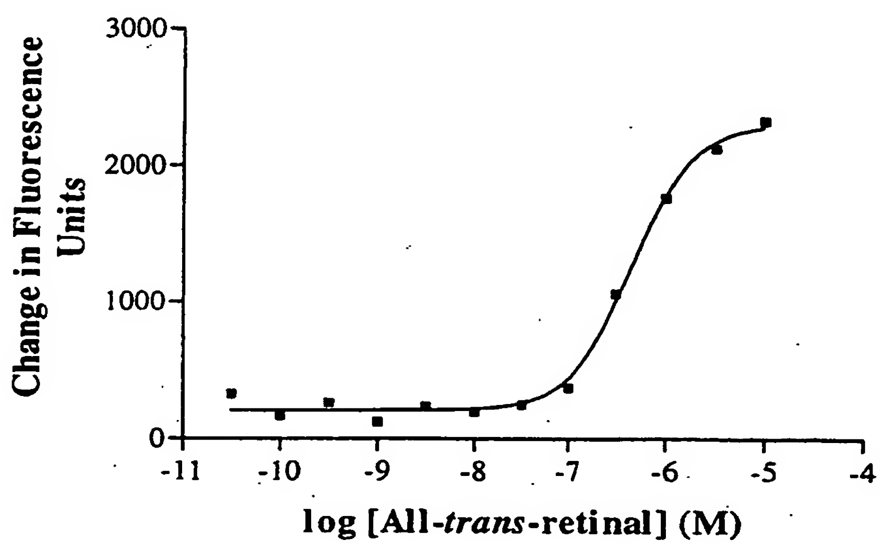


FIGURE 16A

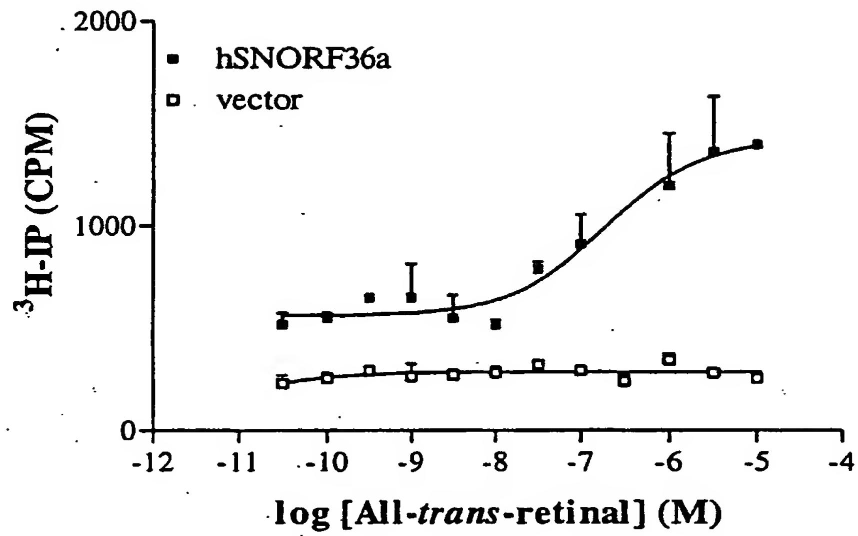


FIGURE 16B

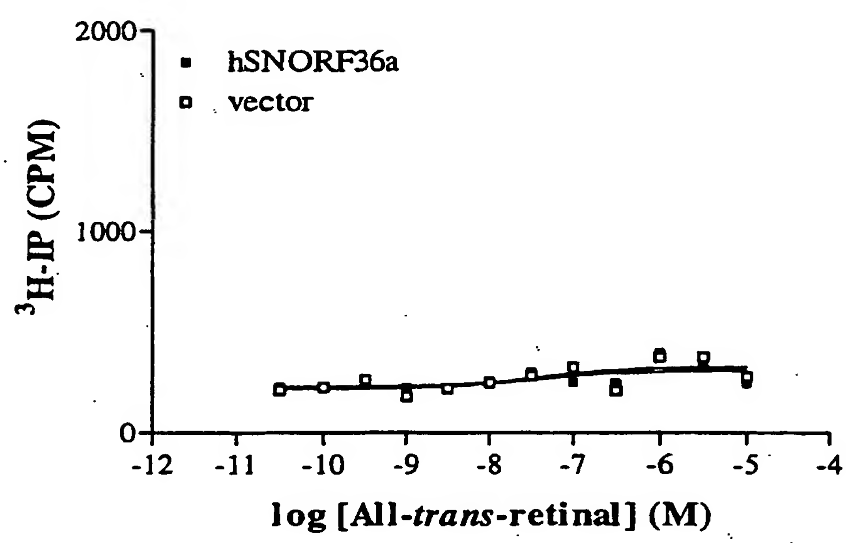


FIGURE 17A

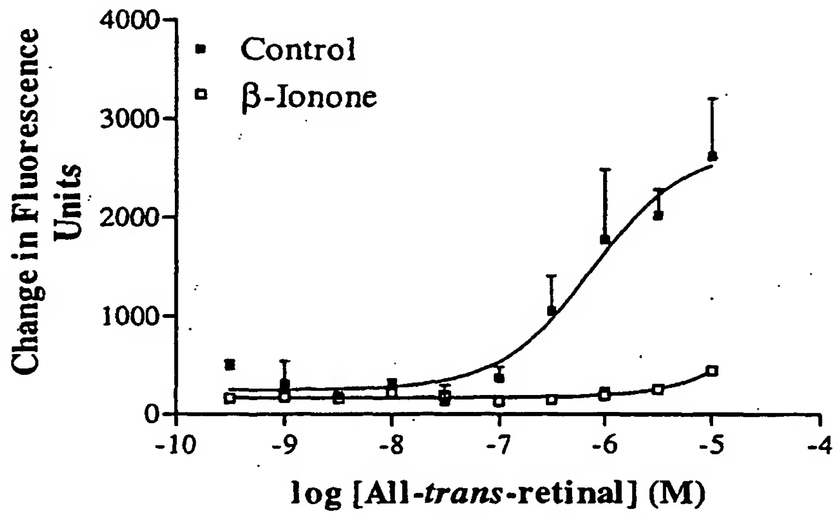


FIGURE 17B

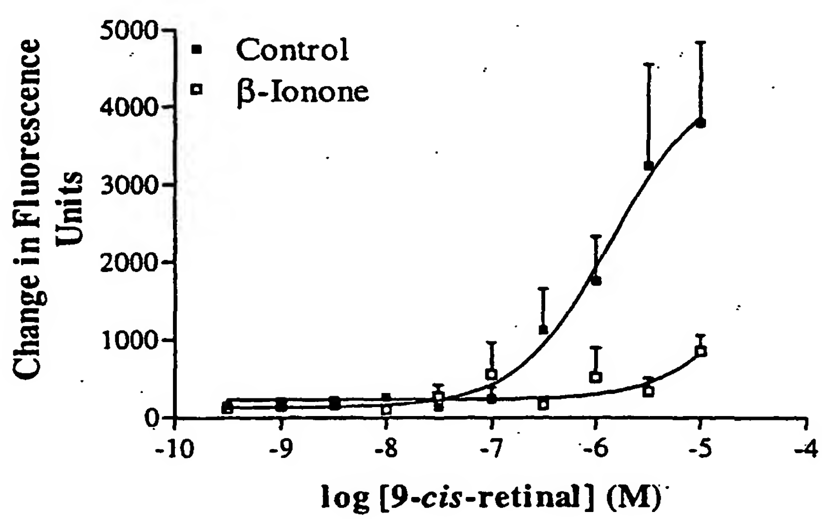


FIGURE 18A

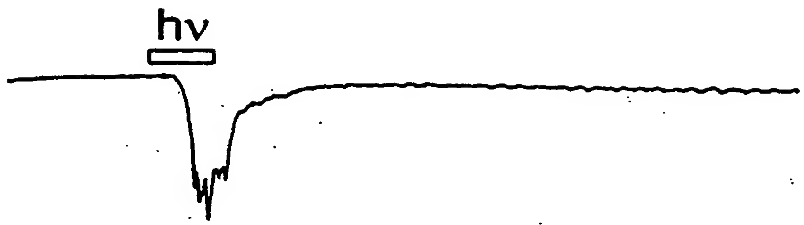


FIGURE 18B

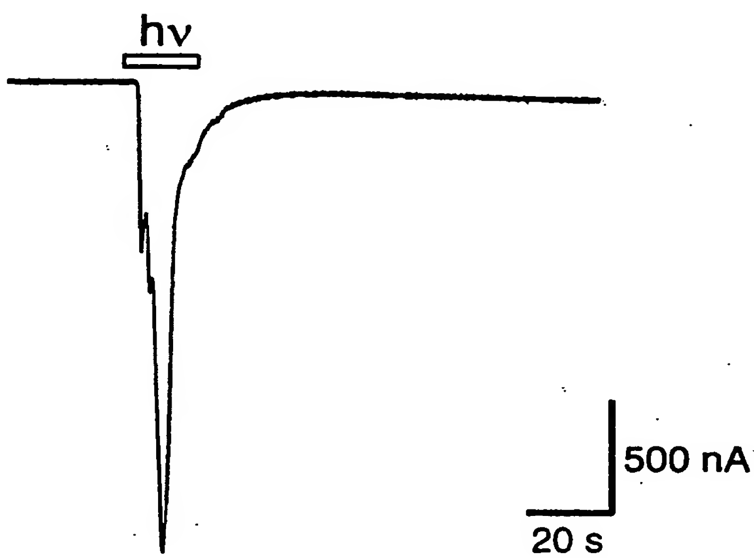
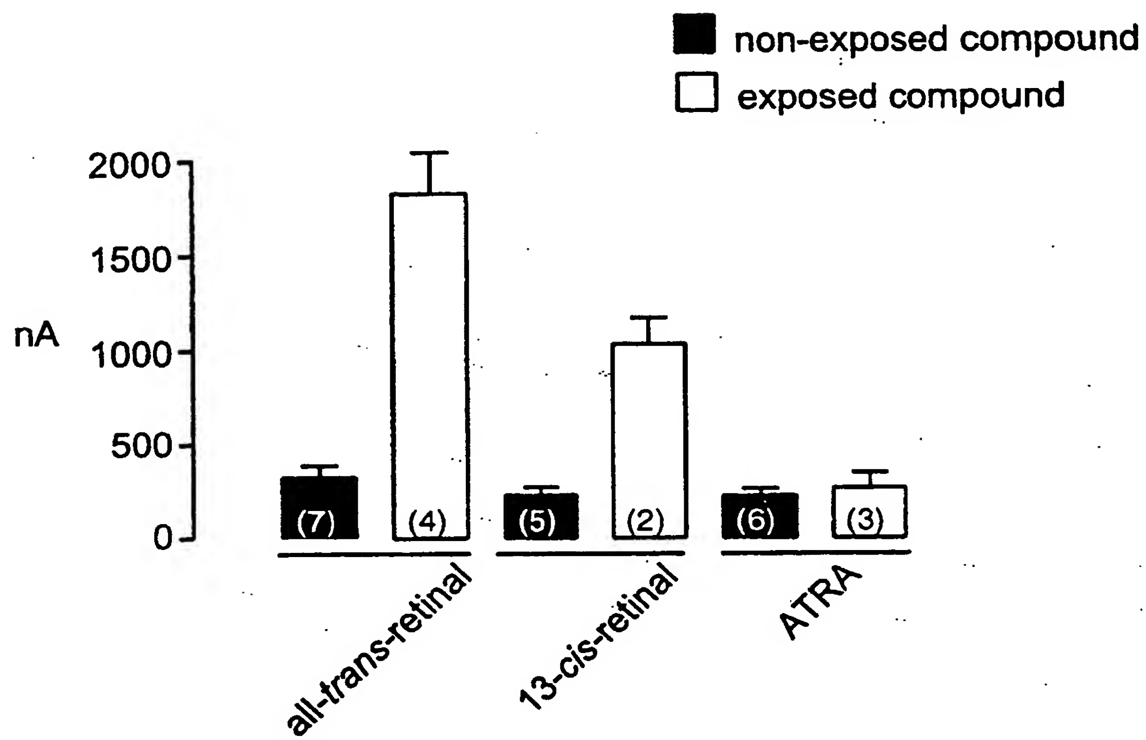


FIGURE 18C

Figure 19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(4)
(5)
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(3)
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ATRA

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<210> 2
<211> 478
<212> PRT
<213> Homo sapiens

<400> 2

Met Asn Pro Pro Ser Gly Pro Arg Val Pro Pro Ser Pro Thr Gln Glu
1 5 10 15

Pro Ser Cys Met Ala Thr Pro Ala Pro Pro Ser Trp Trp Asp Ser Ser
20 25 30

Gln Ser Ser Ile Ser Ser Leu Gly Arg Leu Pro Ser Ile Ser Pro Thr
35 40 45

Ala Pro Gly Thr Trp Ala Ala Ala Trp Val Pro Leu Pro Thr Val Asp
50 55 60

Val Pro Asp His Ala His Tyr Thr Leu Gly Thr Val Ile Leu Leu Val
65 70 75 80

Gly Leu Thr Gly Met Leu Gly Asn Leu Thr Val Ile Tyr Thr Phe Cys
85 90 95

Arg Ser Arg Ser Leu Arg Thr Pro Ala Asn Met Phe Ile Ile Asn Leu
100 105 110

Ala Val Ser Asp Phe Leu Met Ser Phe Thr Gln Ala Pro Val Phe Phe
115 120 125

Thr Ser Ser Leu Tyr Lys Gln Trp Leu Phe Gly Glu Thr Gly Cys Glu
130 135 140

Phe Tyr Ala Phe Cys Gly Ala Leu Phe Gly Ile Ser Ser Met Ile Thr
145 150 155 160

Leu Thr Ala Ile Ala Leu Asp Arg Tyr Leu Val Ile Thr Arg Pro Leu
165 170 175

Ala Thr Phe Gly Val Ala Ser Lys Arg Arg Ala Ala Phe Val Leu Leu
180 185 190

Gly Val Trp Leu Tyr Ala Leu Ala Trp Ser Leu Pro Pro Phe Phe Gly
195 200 205

Trp Ser Ala Tyr Val Pro Glu Gly Leu Leu Thr Ser Cys Ser Trp Asp
210 215 220

Tyr Met Ser Phe Thr Pro Ala Val Arg Ala Tyr Thr Met Leu Leu Cys
225 230 235 240

Cys Phe Val Phe Phe Leu Pro Leu Leu Ile Ile Ile Tyr Cys Tyr Ile
245 250 255

Phe Ile Phe Arg Ala Ile Arg Glu Thr Gly Arg Ala Leu Gln Thr Phe
260 265 270

Gly Ala Cys Lys Gly Asn Gly Glu Ser Leu Trp Gln Arg Gln Arg Leu
275 280 285

Gln Ser Glu Cys Lys Met Ala Lys Ile Met Leu Leu Val Ile Leu Leu
290 295 300

Phe Val Leu Ser Trp Ala Pro Tyr Ser Ala Val Ala Leu Val Ala Phe
305 310 315 320

Ala Gly Tyr Ala His Val Leu Thr Pro Tyr Met Ser Ser Val Pro Ala
325 330 335

Val Ile Ala Lys Ala Ser Ala Ile His Asn Pro Ile Ile Tyr Ala Ile
340 345 350

Thr His Pro Lys Tyr Arg Val Ala Ile Ala Gln His Leu Pro Cys Leu
355 360 365

Gly Val Leu Leu Gly Val Ser Arg Arg His Ser Arg Pro Tyr Pro Ser
370 375 380

Tyr Arg Ser Thr His Arg Ser Thr Leu Thr Ser His Thr Ser Asn Leu
385 390 395 400

Ser Trp Ile Ser Ile Arg Arg Arg Gln Glu Ser Leu Gly Ser Glu Ser
405 410 415

Glu Val Gly Trp Thr His Met Glu Ala Ala Ala Val Trp Gly Ala Ala
420 425 430

Gln Gln Ala Asn Gly Arg Ser Leu Tyr Gly Gln Gly Leu Glu Asp Leu
435 440 445

Glu Ala Lys Ala Pro Pro Arg Pro Gln Gly His Glu Ala Glu Thr Pro
450 455 460

Gly Lys Thr Lys Gly Leu Ile Pro Ser Gln Asp Pro Arg Met
465 470 475

<210> 3
<211> 1541
<212> DNA
<213> Homo sapiens

<400> 3
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agcctgggcc ggcttccatc catcagtccc acagcacctg ggacttgggc tgctgcctgg 180
gtcccccctcc ccacggttga tgttccagac catgcccact ataccctggg cacagtgatc 240
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gctgtgcttc gtggagtcac tgtgatgatg cagagcagaa gcctccggac acctgccaac 360
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<210> 4
<211> 489
<212> PRT
<213> Homo sapiens

<400> 4

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Met Asn Pro Pro Ser Gly Pro Arg Val Pro Pro Ser Pro Thr Gln Glu
  1               5                  10                  15

Pro Ser Cys Met Ala Thr Pro Ala Pro Pro Ser Trp Trp Asp Ser Ser
            20                  25                  30

Gln Ser Ser Ile Ser Ser Leu Gly Arg Leu Pro Ser Ile Ser Pro Thr
        35                  40                  45

Ala Pro Gly Thr Trp Ala Ala Ala Trp Val Pro Leu Pro Thr Val Asp
    50                  55                  60

Val Pro Asp His Ala His Tyr Thr Leu Gly Thr Val Ile Leu Leu Val
65                  70                  75                      80

Gly Leu Thr Gly Met Leu Gly Asn Leu Thr Val Ile Tyr Thr Phe Cys
                85                  90                  95

Arg Ala Val Leu Arg Gly Val Thr Val Met Met Gln Ser Arg Ser Leu
            100                 105                 110

Arg Thr Pro Ala Asn Met Phe Ile Ile Asn Leu Ala Val Ser Asp Phe
        115                 120                 125

Leu Met Ser Phe Thr Gln Ala Pro Val Phe Phe Thr Ser Ser Leu Tyr
    130                 135                 140

Lys Gln Trp Leu Phe Gly Glu Thr Gly Cys Glu Phe Tyr Ala Phe Cys
145                 150                 155                     160

Gly Ala Leu Phe Gly Ile Ser Ser Met Ile Thr Leu Thr Ala Ile Ala
                165                 170                 175

Leu Asp Arg Tyr Leu Val Ile Thr Arg Pro Leu Ala Thr Phe Gly Val
            180                 185                 190

Ala Ser Lys Arg Arg Ala Ala Phe Val Leu Leu Gly Val Trp Leu Tyr
        195                 200                 205

Ala Leu Ala Trp Ser Leu Pro Pro Phe Phe Gly Trp Ser Ala Tyr Val
    210                 215                 220

Pro Glu Gly Leu Leu Thr Ser Cys Ser Trp Asp Tyr Met Ser Phe Thr
225                 230                 235                     240

Pro Ala Val Arg Ala Tyr Thr Met Leu Leu Cys Cys Phe Val Phe Phe
                245                 250                 255
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Leu Pro Leu Leu Ile Ile Ile Tyr Cys Tyr Ile Phe Ile Phe Arg Ala
260 265 270

Ile Arg Glu Thr Gly Arg Ala Leu Gln Thr Phe Gly Ala Cys Lys Gly
275 280 285

Asn Gly Glu Ser Leu Trp Gln Arg Gln Arg Leu Gln Ser Glu Cys Lys
290 295 300

Met Ala Lys Ile Met Leu Leu Val Ile Leu Leu Phe Val Leu Ser Trp
305 310 315 320

Ala Pro Tyr Ser Ala Val Ala Leu Val Ala Phe Ala Gly Tyr Ala His
325 330 335

Val Leu Thr Pro Tyr Met Ser Ser Val Pro Ala Val Ile Ala Lys Ala
340 345 350

Ser Ala Ile His Asn Pro Ile Ile Tyr Ala Ile Thr His Pro Lys Tyr
355 360 365

Arg Val Ala Ile Ala Gln His Leu Pro Cys Leu Gly Val Leu Leu Gly
370 375 380

Val Ser Arg Arg His Ser Arg Pro Tyr Pro Ser Tyr Arg Ser Thr His
385 390 395 400

Arg Ser Thr Leu Thr Ser His Thr Ser Asn Leu Ser Trp Ile Ser Ile
405 410 415

Arg Arg Arg Gln Glu Ser Leu Gly Ser Glu Ser Glu Val Gly Trp Thr
420 425 430

His Met Glu Ala Ala Ala Val Trp Gly Ala Ala Gln Gln Ala Asn Gly
435 440 445

Arg Ser Leu Tyr Gly Gln Gly Leu Glu Asp Leu Glu Ala Lys Ala Pro
450 455 460

Pro Arg Pro Gln Gly His Glu Ala Glu Thr Pro Gly Lys Thr Lys Gly
465 470 475 480

Leu Ile Pro Ser Gln Asp Pro Arg Met
485

<210> 5
<211> 250

<212> DNA
<213> Rattus norvegicus

<400> 5
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caagagacgg acggcactag tcctgctagg tgtctggctc tatgccctgg cctggagtct 120
gccgcctttc tttggctgga gcgcctacgt gcccgagggg ctgctgacat cctgctcctg 180
ggactacgtg accttcacgc ccctcgtgcg cgcctacacc atgctgctct tctgctttgt 240
cttcttcctc 250

<210> 6
<211> 83
<212> PRT
<213> Rattus norvegicus

<400> 6
Ile Ala Met Asp Arg Tyr Leu Val Ile Thr Arg Pro Leu Ala Thr Ile
1 5 10 15

Gly Met Arg Ser Lys Arg Arg Thr Ala Leu Val Leu Leu Gly Val Trp
20 25 30

Leu Tyr Ala Leu Ala Trp Ser Leu Pro Pro Phe Phe Gly Trp Ser Ala
35 40 45

Tyr Val Pro Glu Gly Leu Leu Thr Ser Cys Ser Trp Asp Tyr Val Thr
50 55 60

Phe Thr Pro Leu Val Arg Ala Tyr Thr Met Leu Leu Phe Cys Phe Val
65 70 75 80

Phe Phe Leu

<210> 7
<211> 1473
<212> DNA
<213> Rattus norvegicus

<400> 7
tttaagtcct ccaagagcct gagcatgaac tctccttcag aatcaagagt cccttcaagc 60
ttaactcagg atcccagctt taccgccagc cctgccctcc tacaaggcat ttggaacagc 120
actcagaaca tctccgtcag agtccagctt ctatccgtta gccccacgac acctgggctt 180
caggctgctg cctgggtccc cttccccaca gtcgacgtcc cagatcatgc tcactatacc 240
ctaggcacgg tgatcctgct ggtgggactc acagggatgc tgggtaacct gacagtcatc 300
tacaccttct gcaggaatag aggcctgcgg acaccggcaa acatgctcat catcaacctg 360

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tacaagaagt ggctcttcgg ggagacaggt tgcaagttct atgccttctg tggggctgtc 480
tttggcatcg tttccatgat caccctgaca gccatagcca tggaccgcta tctggtgatc 540
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<210> 8
<211> 474
<212> PRT
<213> Rattus norvegicus

<400> 8

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Met Asn Ser Pro Ser Glu Ser Arg Val Pro Ser Ser Leu Thr Gln Asp
 1               5                  10                  15

Pro Ser Phe Thr Ala Ser Pro Ala Leu Leu Gln Gly Ile Trp Asn Ser
            20                  25                  30

Thr Gln Asn Ile Ser Val Arg Val Gln Leu Leu Ser Val Ser Pro Thr
        35                  40                  45

Thr Pro Gly Leu Gln Ala Ala Ala Trp Val Pro Phe Pro Thr Val Asp
    50                  55                  60

Val Pro Asp His Ala His Tyr Thr Leu Gly Thr Val Ile Leu Leu Val
65                  70                  75                  80

Gly Leu Thr Gly Met Leu Gly Asn Leu Thr Val Ile Tyr Thr Phe Cys
                85                  90                  95

Arg Asn Arg Gly Leu Arg Thr Pro Ala Asn Met Leu Ile Ile Asn Leu
            100                 105                 110
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Ala Val Ser Asp Phe Leu Met Ser Phe Thr Gln Ala Pro Val Phe Phe
115 120 125

Ala Ser Ser Leu Tyr Lys Lys Trp Leu Phe Gly Glu Thr Gly Cys Lys
130 135 140

Phe Tyr Ala Phe Cys Gly Ala Val Phe Gly Ile Val Ser Met Ile Thr
145 150 155 160

Leu Thr Ala Ile Ala Met Asp Arg Tyr Leu Val Ile Thr Arg Pro Leu
165 170 175

Ala Thr Ile Gly Met Arg Ser Lys Arg Arg Thr Ala Leu Val Leu Leu
180 185 190

Gly Val Trp Leu Tyr Ala Leu Ala Trp Ser Leu Pro Pro Phe Phe Gly
195 200 205

Trp Ser Ala Tyr Val Pro Glu Gly Leu Leu Thr Ser Cys Ser Trp Asp
210 215 220

Tyr Val Thr Phe Thr Pro Leu Val Arg Ala Tyr Thr Met Leu Leu Phe
225 230 235 240

Cys Phe Val Phe Phe Leu Pro Leu Leu Ile Ile Ile Phe Cys Tyr Ile
245 250 255

Phe Ile Phe Arg Ala Ile Arg Glu Thr Gly Arg Ala Cys Glu Gly Cys
260 265 270

Gly Glu Ser Pro Leu Arg Arg Arg Gln Trp Gln Arg Leu Gln Ser Glu
275 280 285

Trp Lys Met Ala Lys Val Ala Leu Ile Val Ile Leu Leu Phe Val Leu
290 295 300

Ser Trp Ala Pro Tyr Ser Thr Val Ala Leu Val Gly Phe Ala Gly Tyr
305 310 315 320

Ser His Ile Leu Thr Pro Tyr Met Ser Ser Val Pro Ala Val Ile Ala
325 330 335

Lys Ala Ser Ala Ile His Asn Pro Ile Ile Tyr Ala Ile Thr His Pro
340 345 350

Lys Tyr Arg Ala Ala Ile Ala Gln His Leu Pro Cys Leu Gly Val Leu
355 360 365

```
Leu Gly Val Ser Gly Gln Arg Ser His Pro Ser Leu Ser Tyr Arg Ser
    370                 375                 380

Thr His Arg Ser Thr Leu Ser Ser Gln Ser Ser Asp Leu Ser Trp Ile
385                 390                 395                     400

Ser Gly Gln Lys Arg Gln Glu Ser Leu Gly Ser Glu Ser Glu Val Gly
                405                 410                     415

Trp Thr Asp Thr Glu Thr Thr Ala Ala Trp Gly Ala Ala Gln Gln Ala
            420                 425                 430

Ser Gly Gln Ser Phe Cys Ser His Asp Leu Glu Asp Gly Glu Val Lys
        435                 440                 445

Ala Pro Ser Ser Pro Gln Glu Gln Lys Ser Lys Thr Pro Lys Thr Lys
    450                 455                 460

Arg His Leu Pro Ser Leu Asp Arg Arg Met
465                 470
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<210> 9
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 9
catcgccctc gacgtgctgt gctgcacctc atccatcttg cacct 45

<210> 10
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 10
catggacagg tcgcgctacc gcgtgtccac gttctaccta ctcca 45

<210> 11
<211> 45

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 11
ggcatcatca tgggcacctt catcctctgc tggctgccct tcttc 45

<210> 12
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 12
gcagaagggc agaacaagag ccacgatgaa gaagggcagc cagca 45

<210> 13
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 13
tggctgtcat cggacatcac ttgttgcact gcctccatcc tgcac 45

<210> 14
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 14
gtagcggtcc agggcgatga cacagaggtg caggatggag gcagt 45

<210> 15
<211> 27

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 15
ccagccgaag aagggtggca gactcca 27

<210> 16
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 16
cttctaggcc tgtacggaag tgtta 25

<210> 17
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 17
gcacaggctg cgagttctat tcctt 25

<210> 18
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 18
ctggtaatca cacacccgct ggccaccttt ggtgtggcgt ccaag 45

<210> 19
<211> 24

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 19
agatcatgct gctggtcatc ctcc 24

<210> 20
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 20
tcgtgctctc ctgggctccc t 21

<210> 21
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 21
tcctccatga tcaccctgac ggc 23

<210> 22
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 22
tctggagagc ccgtcctgtc tcc 23

<210> 23
<211> 48

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 23
cggccgtgcg tgcctacacc atgcttctct gctgcttcgt gttcttcc 48

<210> 24
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 24
ttggacgcca caccaaaggt ggcc 24

<210> 25
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 25
ggtatagatg accgtcaggt tgcc 24

<210> 26
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 26
cgaacaggat cctctctgtg ggctcgagca aggacc 36

<210> 27
<211> 24

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 27
acgtgtgcgt acccagcaaa ggcc 24

<210> 28
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 28
gtcccacagc acctgggact tgggctgc 28

<210> 29
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 29
gcagcccaag tcccaggtgc tgtgggac 28

<210> 30
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 30
ggcaacctga cggtcatcta tacc 24

<210> 31
<211> 35

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 31
cagcataagc ttccagtggg cgtcctacat cctgg 35

<210> 32
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 32
cagtagatga tgataagcag agg 23

<210> 33
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 33
cgaacaggat cccatagcca tggaccgcta tctgg 35

<210> 34
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 34
cctagcaagc ttgaggaaga agacaaagca gaagagc 37

<210> 35
<211> 48

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 35
cggacggcac tagtcctgct aggtgtctgg ctctatgccc tggcctgg 48

<210> 36
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 36
ggctgcgagt tctatgcctt 20

<210> 37
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 37
ttaccaggta gcggtccagg 20

<210> 38
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 38
agctctcttt ggcatttcct ccatgatca 29

<210> 39
<211> 18

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 39
ctgggcaacc tgacggtc 18

<210> 40
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 40
caggtgtccg gaggcttct 19

<210> 41
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 41
tgtgcttcgt ggagtcactg tgatgat 27

<210> 42
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 42
tccactggcc accatcg 17

<210> 43
<211> 22

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 43
gggcatagag ccagacacct ag 22

<210> 44
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 44
catgagatcc aagagacgga cggca 25

<210> 45
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 45
tcctccatga tcaccctgac ggc 23

<210> 46
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 46
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<210> 47
<211> 24

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 47
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<210> 48
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer/probe

<400> 48
ttggacgcca caccaaaggt ggcc 24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/12065

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C07K 14/705; C07H 21/04; C12N 15/63, 1/21; C12P 21/02; G01N 33/53
US CL : 530/350; 536/23.5; 436/7.1, 69.1, 252.3, 254.2, 320.1, 361
According to International Patent Classification (IPC) r to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 536/23.5; 436/7.1, 69.1, 252.3, 254.2, 320.1, 361

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Commercial Sequence Databases: GenEmbl, N_Geneseq_36, Issued_Patents_NA, EST, A_Geneseq_36, Issued Patents_AA, PIR_64, SwissProt_38, STREMBL_12

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROVENCIO, I. et al. Melanopsin: An opsin in Melanophores, Brain and Eye. Proc. Ntl. Acad. Sci. USA. January 1998. Vol. 95, pages 340-345, see entire article, especially Fig. 3.	12-14
A,P	US 6,008,338 A (Fong) 28 December 1999, see entire article.	1-43, 63-74, 76-80, 82-90, 92-98, 100, 115-119, 124-137, 143-149

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 17 AUGUST 2000	Date of mailing of the international search report 07 SEP 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer EILEEN B. O'HARA Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/12065

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-43, 63-74, 76-80, 82-90, 92-98, 100, 115-119, 124-137 and 143-149

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-43, 63-74, 76-80, 82-90, 92-98, 100, 115-119, 124-137 and 143-149, drawn to nucleic acids of SNORF36 receptor, vectors, host cells, SNORF36 protein, and method of making protein recombinantly and a process to screen for compounds that bind to or are antagonists or agonists of SNORF36 receptor and a method for detecting SNORF36 protein with antibody.
Group II, claim(s) 44-46 and 51-55, drawn to antisense oligonucleotides.
Group III, claim(s) 47, 48, 50 and 56, drawn to antibodies.
Group IV, claims 57, 60,61 and 62, drawn to a transgenic mammal expressing DNA encoding SNORF35 receptor.
Group V, claim(s) 58, 60, 61 and 62, drawn to a transgenic mammal comprising a homologous recombination knockout of SNORF36 receptor.
Group VI, claim(s) 59 and 62, drawn to a transgenic mammal expressing antisense DNA complementary to the DNA encoding a mammalian SNORF36 receptor.
Group VII, claim(s)49, 75, 81, 91, 105, 106, 122, 123, 141, 142 and 151, drawn to a compound that binds to SNORF36 receptor which could be an antagonist.
Group VIII, claim(s) 49, 110, 111, 120, 121, 138-140 and 150, drawn to a compound that binds to SNORF36 receptor which could be an agonist.
Group IX, claim(s) 99, 113 and 114, drawn to a method of detecting SNORF36 mRNA or DNA or a method of diagnosing a predisposition to a disorder, by nucleic acid hybrization.
Group X, claim(s)101 and 102, drawn to a method of determing the physiological effects of varying levels of mammalian SNORF36 using a transgenic mammal.
Group XI, claim(s) 103, 104, 108 and 109, drawn to a method for identifying agonists or antagonists of SNORF36 using transgenic mammals.
Group XII, claims 107 and 153, drawn to a method of treating a subject by administering a SNORF36 antagonist.
Group XIII, claims 112 and 152, drawn to a method of treating a subject by administering a SNORF36 agonist.
Group XIV, claims 154-165, drawn to a process of making a composition of matter which binds to a SNORF36 receptor.

The inventions listed as Groups do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Pursuant to 37 C.F.R. § 1.475(d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first recited product, polynucleotides encoding SNORF36 receptor proteins, vectors, host cells, SNORF36 polypeptides, a method of screening for compounds that bind to SNORF36 receptor, a method of detecting SNORF36 polypeptides and a method of recombinantly producing SNORF36. Further pursuant to 37 C.F.R. § 1.475(d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.